VIASURE

Real Time PCR Detection Kits

by CerTest BIOTEC

SARS-CoV-2, Flu (A+B) & RSV

Handbook for the following references/

Håndbog til følgende referencer:

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System **BD REF 444217**

to be used with the BD MAX™ System

til brug sammen med BD MAX™ System

ENGLISH

1. Intended use

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is an automated real-time RT-PCR test designed for the qualitative detection and differentiation of RNA from the SARS-CoV-2, Influenza A (Flu A), Influenza B (Flu B) and/or Human Respiratory Syncytial Virus A/B (RSV) in respiratory samples from individuals suspected of COVID-19 or other respiratory infection by their healthcare provider. This test is intended to be used as an aid in the identification of the presence of the SARS-CoV-2, Flu A, Flu B and/or RSV viral RNA. The assay uses the BD MAX™ System for automated extraction of RNA and subsequent real-time RT-PCR employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. RNA is extracted from respiratory specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes specific for SARS-CoV-2, Flu A, Flu B and/or RSV.

2. Summary and Explanation

Coronavirus are enveloped non-segmented positive-sense RNA viruses and belong to *Coronaviridae* family. There are six coronavirus species known to cause human diseases. Four viruses (229E, OC43, NL63 and HKU1) cause common cold symptoms and the other two (severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)) are zoonotic and producing more severe complications. SARS-CoV and MERS-CoV have caused more than 10,000 cumulative cases in the past two decades, with mortality rates of 34% MERS-CoV and 10% SARS-CoV.

In December 2019, some people that worked at or lived around the Huanan seafood market in Wuhan, Hubei Province, China, have presented pneumonia of unknown cause. Deep sequencing analysis of the respiratory samples indicated a novel coronavirus, which was named firstly 2019 novel coronavirus (2019-nCoV) and lately SARS-CoV-2.

Human-to-human transmission of the SARS-CoV-2 has been confirmed, even in the incubation period without symptoms, and the virus causes severe respiratory illness like those SARS-CoV produced. Although the pneumonia is the principal illness associated, a few patients have developed severe pneumonia, pulmonary edema, acute respiratory distress syndrome, or multiple organ failure and death. Centers of Disease Control and Prevention (CDC) believes that symptoms of SARS-CoV-2 may appear in as few as 2 days or as long as 14 days after exposure, being the most common fever or chills, cough, fatigue, anorexia, myalgia and dyspnea. Less common symptoms are sore throat, nasal congestion, headache, diarrhea, nausea and vomiting. Loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms has also been reported. Older adults and people who have severe underlying medical conditions like heart or lung disease or diabetes seem to be at higher risk for developing more serious complications from COVID-19 illness.

CDC recommends upper respiratory tract specimens (nasopharyngeal (NP) and oropharyngeal (OP) swabs, nasal mid-turbinate swab, nasal swab, nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimens collected mainly by a healthcare provider) and/or lower respiratory specimens (sputum, endotracheal aspirate, or

bronchoalveolar lavage in patients with more severe respiratory disease) for the identification of SARS-CoV-2 and other respiratory viruses, such as Influenza and RSV.

Influenza viruses belong to the *Orthomyxoviridae* family and cause the majority of viral lower respiratory tract infections. Influenza A and B are a significant cause of morbidity and mortality worldwide, considering that elderly and compromised individuals are especially at risk of developing severe illness and complications such as pneumonia. People feel some or all of these symptoms: fever or feeling feverish/chills, cough, sore throat, nasal stuffiness and discharge, myalgia, headaches, and anorexia. The influenza viruses can be spread from person to person in two different ways: through the air (large droplets and aerosols from sneezing and coughing), and by direct or indirect contact.

Influenza A and B are an enveloped, single stranded RNA viruses that containing eight segmented strands of genome RNA, which typically encodes 11 or 12 viral proteins. The viral envelope, derived from the host plasma membrane, consists of a lipid bilayer containing transmembrane proteins, like hemagglutinin (HA) and neuraminidase (NA), and matrix proteins M1 and M2. Influenza A viruses are further classified into subtypes based on the antigenicity of their "HA" and "NA" molecules, whereas Influenza B is divided into 2 antigenically and genetically distinct lineages, Victoria and Yamagata.

Human respiratory syncytial viruses A and B (RSV) belong to the *Paramyxoviridae* family and are the most important viral agents of acute respiratory infections. RSV is an enveloped, nonsegmented, negative, single stranded linear RNA genome virus. Respiratory syncytial virus is a common contributor of respiratory infections causing bronchitis, pneumonia, and chronic obstructive pulmonary infections in people of all ages. People often feel some or all of these symptoms: rhinorrhea, low-grade fever, cough, sore throat, headache, and wheezing. RSV is transmitted via large nasopharyngeal secretion droplets from infected individuals, close contact, or self-inoculation after touching contaminated surfaces.

Diagnosis can be problematic, as a wide range of pathogens can cause acute respiratory infections presenting with similar clinical syndromes. Real-time PCR assays have been shown to be a sensitive and specific diagnostic tool for the detection of SARS-CoV-2, Flu A, Flu B and RSV viruses.

3. Principle of the procedure

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is designed for the identification of SARS-CoV-2, Flu A, Flu B and /or RSV in respiratory samples. The detection is done in one step real-time RT-PCR format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction tube. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of two conserved regions of *N* gene (N1 and N2) for SARS-CoV-2, a conserved region of the *M1* gene for Flu A and Flu B, and a conserved region of the *N* gene for RSV using specific primers and fluorescent-labeled probes.

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is based on 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the

fluorescent signal which is proportional to the quantity of the target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System is composed of two different reaction tubes. One of the tubes detects and differentiates the RNA from Flu A, Flu B and/or RSV (Transparent Red or 1A foil) and the other tube detects specifically the RNA from SARS-CoV-2 (Transparent Green or 1G foil). Each tube contains all the components necessary for real-time PCR assay (specific primers/probes, dNTPS, buffer, polymerase, reverse-transcriptase) in a stabilized format, as well as an internal control (endogenous in the SARS-CoV-2 reaction tube) to monitor the extraction process and/or inhibition of the polymerase activity. The SARS-CoV-2 assay uses a human housekeeping gene as an endogenous Internal Control (human *RNase P* gene). Human housekeeping genes are involved in basic cell maintenance and, therefore, are expected to be present in all nucleated human cells and maintain relatively constant expression levels. Each RNA targets are amplified and detected in specific channels (475/520, 585/630, and/or 630/665) and the internal control (IC) in channel 530/565. In the Flu A, Flu B and/or RSV assay, Flu A RNA target is amplified and detected in channel 475/520, Influenza B RNA target in channel 585/630, RSV RNA target in channel 630/665 and the internal control (IC) of this assay in channel 530/565. In SARS-CoV-2 assay, N2 target is amplified and detected in channel 475/520, N1 target in channel 630/665 and the endogenous internal control (IC) in channel 530/565.

4. Reagents provided

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System includes the following materials and reagents detailed in Table 1:

Reference	Reagent/Material	Description	Color/Barcode	Amount
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and internal control in stabilized format	Transparent Red or 1A foil	2 pouches of 12 tubes
VS-NCO312	*SARS-CoV-2 (N1 + N2)* reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	Transparent Green or 1G foil	2 pouches of 12 tubes
VS-RB09	Rehydration Buffer tube	Solution to reconstitute the stabilized product	Transparent Orange or 11 foil	1 pouch of 24 tubes

Table 1. Reagents and materials provided in VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System with Cat. N°. VS-FNR124 (444217).

5. Reagents and equipment to be supplied by the user

The following list includes the materials and equipment that are required for use but not included in the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System.
- BD MAX™ ExK™ TNA-3 (Ref:442827 or 442828)
- BD MAX™ PCR Cartridges (Ref: 437519)

- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).
- Filter tips.
- Powder-free disposable gloves

6. Transport and storage conditions

- The kits can be shipped and stored at 2-40ºC until the expiration date which is stated on the label.
- After opening the aluminum pouches which contain the reaction tubes can be used up to 28 days.

7. Precautions for users

- The product is indented for use by professional users only, such as laboratory or health professionals and technicians, trained in molecular biological techniques.
- For *in vitro* diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.
- Keep components away from light.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- Make sure to use a tube to determine RNA from Influenza A, Influenza B and RSV in Snap-In 2 (green position) and another tube to determine RNA from SARS-CoV-2 in Snap-In 4 (blue position). Be careful not to mix them throughout the entire process.
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink or smoke in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, storage, treatment and disposal of samples.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Procedure

8.1. SAMPLE COLLECTION, STORAGE AND TRANSPORT

The VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System has been validated on nasopharyngeal/ oropharyngeal swab collected in viral transport media (VTM) (Vircell S.L., Spain).

Other types of samples from nasopharyngeal/oropharyngeal swabs in VTM must be validated by the user.

Collection, storage and transport specimens should be maintained per the conditions validated by the user. Overall, respiratory samples should be collected and labelled appropriately in clean containers with or without transport media (depending on sample type) and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at 2 to 8°C for up to 48 hours, following the local and national regulations for the transport of pathogen material. For long term transport (more than 48 hours), we recommend shipping at ≤-20°C. It is recommended to use fresh specimens for the test. The samples can be stored at 2 to 8°C for up to 48 hours or frozen at -20°C or ideally at -70°C for conservation. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

8.2. SAMPLE PREPARATION AND RNA EXTRACTION

Perform the sample preparation according to the recommendations in the instructions for use of extraction kit used, BD MAX™ ExK™ TNA-3. Note that some other samples may require pre-processing. Application-specific extraction preparation procedures should be developed and validated by the user.

1. Pipette 400 µL of nasopharyngeal/ oropharyngeal swab collected in viral transport media (VTM) into a BD MAX™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

Note: The *Flu A, Flu B & RSV* reaction tube has been validated with a sample volume of 200-400 µL and the *SARS-CoV-2 (N1 + N2)* reaction tube with a sample volume of 400-750 µL.

8.3. PCR PROTOCOL

Note: Please, refer to the BD MAX™ System User's Manual for detailed instructions.

8.3.1. Creating PCR test program for VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System

Note: If you have already created the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection test, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.
3) In the Basic Information tab, within the "Test Name" window, name your test: i.e. VIASURE *SARS-CoV-2, Flu (A+B) & RSV* (VSARSCoV2,FluA+B,RSV).
4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
5) In the "Master Mix Format" drop down menu, choose "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) In the "Sample extraction parameters" select "User defined" and adjust sample volume to 950 µL.
7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".
8) If running software version 5.00 or higher and have barcoded foil snap-in tubes, in the "Custom Barcodes" select the following configuration:
    a. Snap-In 2 Barcode: 1A (concerning *Flu A, Flu B & RSV* reaction tube)
    b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube)
    c. Snap-In 4 Barcode: 1G (concerning *SARS-CoV-2 (N1 + N2)* reaction tube)
9) "PCR Settings" and "Test Steps" must be completed for Snap-In 2 (green) and Snap-In 4 (blue) positions.
10) Snap-In 2 (green). In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 2).

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	Flu A	60	100	0	40
530/565 (HEX)	IC	80	300	0	40
585/630 (ROX)	Flu B	60	200	0	40
630/665 (Cy5)	RSV	60	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Table 2. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

11) Snap-In 2 (green). In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 3), as well.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	0.0	0.0	0.0	0.0
	530/565	0.0	-	2.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0
	630/665	0.0	0.0	4.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Table 3. Spectral cross-talk parameters.

12) Snap-In 2 (green). In "Test Steps" tab, enter the PCR protocol (Table 4).

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Reverse transcription	Hold	1	900	45°C	-
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			61.1	63°C	✓

Table 4. PCR protocol.

13) Snap-In 4 (blue). In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 5).

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	SARS-CoV-2 N2 target	80	150	0	40
530/565 (HEX)	Endogenous IC	80	150	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	SARS-CoV-2 N1 target	80	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Table 5. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

14) Snap-In 4 (blue). In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 6), as well.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	3.0	0.0	0.0	0.0
	530/565	1.0	-	0.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0
	630/665	0.0	0.0	0.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Table 6. Spectral cross-talk parameters.

15) Snap-In 4 (blue). In "Test Steps" tab, enter the PCR protocol (Table 7).

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Reverse transcription	Hold	1	900	45°C	-
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			61.1	63°C	✓

Table 7. PCR protocol.

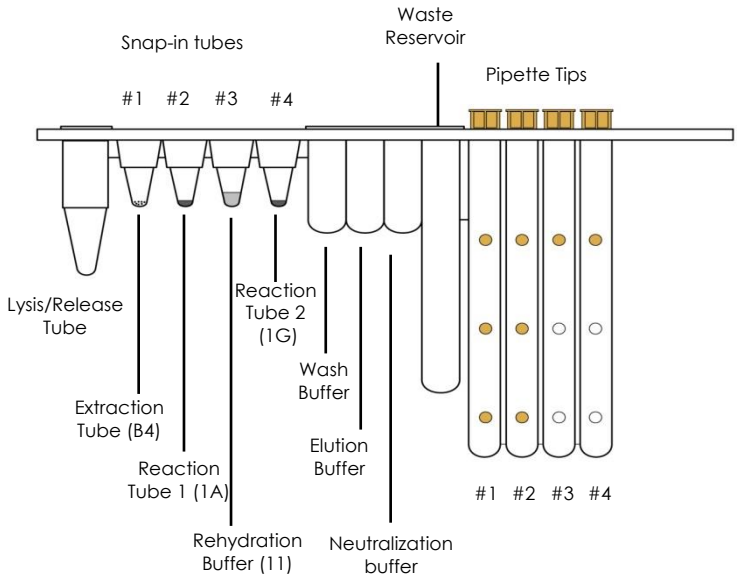
16) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

1) For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK TNA-3 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
2) Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip (Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.
3) Determine and separate the appropriate number of *Flu A, Flu B & RSV* reaction tubes (red or 1A foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
4) Remove the required number of Rehydration Buffer tubes (orange or 11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air, and close the pouch with the zip seal.
    a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.

5) Determine and separate the appropriate number of *SARS-CoV-2 (N1 + N2)* reaction tubes (green or 1G foil) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.

Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK TNA-3 kit.



8.3.3. BD MAX™ Instrument set up

1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
2) In the "Test" drop down menu, select VSARSCoV2,FluA+B,RSV (if not already created see Section 8.3.1).
3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull down menu (optional).
4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Worklist, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID and/or Accession window of the Worklist and click the "Save" button. Continue until all Sample Buffer Tubes are entered. Ensure that the specimen/patient ID and the Sample Buffer Tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click "Start Run" to begin the procedure.

8.3.4 BD MAX™ report

1) In main menu, click the "Results" button.
2) Either double click on your run in the list or press the "view button".
3) Click on "Print", select: "Run Details, Test Details and Plot…"
4) Click on "Print or Export button" on the "Run Reports" screen.

9. Result interpretation

For a detailed description on how to analyze data, refer to the BD MAX™ System User's manual.

The analysis of the data is done by the BD MAX™ software according to the manufacturer´s instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each sample tested in the following way:

- Ct value of 0 indicates that there was no Ct value calculated by the software with the specified Threshold (see Table 2). Amplification curve of the sample showing a "0" Ct value must be checked manually.

- Ct value of -1 indicates that no amplification process has occurred.

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the sample interpretation guidelines outlined in Tables 8 and 9.

Check Internal Control signal to verify the correct functioning of the amplification mix. In addition, check that there is no report of BD MAX™ System failure.

Results should be read and analyzed using the following tables:

a. *Flu A, Flu B & RSV* reaction tube: Snap-In 2

Flu A (475/520)	Flu B (585/630)	RSV (630/665)	Internal control (530/565)	Interpretation
+	+	+	+/-[1]	**Flu A, Flu B and RSV RNA Detected[1]**
+	-	-	+/-[1]	**Flu A RNA Detected, Flu B and RSV RNA Not Detected[1]**
+	+	-	+/-[1]	**Flu A and Flu B RNA Detected, and RSV RNA Not Detected[1]**
+	-	+	+/-[1]	**Flu A and RSV RNA Detected, and Flu B RNA Not Detected[1]**
-	+	-	+/-[1]	**Flu B RNA Detected, Flu A and RSV RNA Not Detected[1]**
-	+	+	+/-[1]	**Flu B and RSV RNA Detected, Flu A RNA Not Detected[1]**
-	-	+	+/-[1]	**RSV RNA Detected, Flu A and Flu B RNA Not Detected[1]**
-	-	-	+[2]	**Flu A, Flu B and RSV RNA Not Detected[2]**
-	-	-	-[2]	**Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[2]**
IND	IND	IND	IND	**Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.**
INC	INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 8. Sample interpretation *Flu A, Flu B & RSV* reaction tube

+: Amplification occured
-: No amplification occured

1 A sample is considered positive if the Ct value obtained is less than 40. The internal control may or may not show an amplification signal, because a high copy number of target can cause preferential amplification of target-specific nucleic acids instead of the internal control. In these cases, the detection of the IC is not necessary.

2 A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive (Ct less than 40). An inhibition of the PCR reaction can be excluded by the amplification of internal control. In case of unresolved results (UNR), absence of internal control signal in negative sample it is recommended to repeat the assay.

b. *SARS-CoV-2 (N1 + N2)* reaction tube: Snap-In 4

SARS-CoV-2 (N2 target) (475/520)	Endogenous Internal Control (530/565)	SARS-CoV-2 (N1 target) (630/665)	Interpretation
+	+/-[3]	+	**SARS-CoV-2 *N gene* RNA Detected [3]**
+[4]	+/-[3]	-	**SARS-CoV-2 *N gene* RNA Detected [3,4]**
-	+/-[3]	+[4]	**SARS-CoV-2 *N gene* RNA Detected [3,4]**
-	+[5]	-	**SARS-CoV-2 *N* gene RNA Not Detected[5]**
-	-[5]	-	**Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[5]**
IND	IND	IND	**Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.**
INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 9. Sample interpretation *SARS-CoV-2 (N1 + N2)* reaction tube

+: Amplification occurred

-: No amplification occurred

3 A sample is considered positive if the Ct value obtained is less than 40. The endogenous Internal Control (IC) may or may not show an amplification signal. Sometimes, the IC detection is not necessary because a high copy number of the target can cause preferential amplification of target-specific nucleic acids.

4 If only one target site of the *N* gene amplifies, verify the sigmoid shape of the curve and the intensity of fluorescence. In case of a doubtful interpretation, depending on the available material, it is also recommended to:

a) re-extract and re-test another aliquot of the same specimen (if possible, increase sample volume to 750 µl) or,

b) obtain a new specimen and re-test.

5 In the case of SARS-CoV-2 target sites negative, IC must show an amplification signal with Ct less than 35. The Ct value could be very variable due to the Endogenous Internal Control is a human housekeeping gene that should be present

in all human nucleated cells in the original sample. If there is an absence of signal or Ct value ≥ 35 of the endogenous Internal Control, the result is considered as 'Unresolved', and retesting is required.

In case of a continued ambiguous result, it is recommended to review the instructions for use, the extraction process used by the user; to verify the correct performance of each RT-qPCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence.

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Although this assay can be used with other types of samples it has been validated with nasopharyngeal/ oropharyngeal swab collected in VTM.
- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- The quality of the test depends on the quality of the sample; proper extracted nucleic acid from respiratory samples must be extracted.
- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by SARS-CoV-2, Flu A, Flu B and/or RSV either samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.
- The specific primer and probe combinations for detection of conserved regions of *N gene* (SARS-CoV-2) used in VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System have been designed based on the US CDC assay for specific detection of SARS-CoV-2 by amplifying two unique regions of the N gene. They do not show significant combined homologies with the human genome, human microflora, SARS-CoV or other coronaviruses, which might result in predictable false positive.
- False Negative results may arise from several factors and their combinations, including:
    - Improper specimens' collection, transport, storage, and/or handling methods.
    - Improper processing procedures (including RNA extraction).
    - Degradation of the viral RNA during sample shipping/storage and/or processing.
    - Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown SARS-CoV-2, Flu and/or RSV variants.
    - A viral load in the specimen below the limit of detection for the assay.

  - The presence of RT-qPCR inhibitors or other types of interfering substances.
  - Failure to follow instructions for use and the assay procedure.
- In *SARS-CoV-2 (N1 + N2)* reaction tube, a single-target site amplification or even random positive results is suggestive of slightly different amplification yield of the target site of the *N* gene. Samples with low viral load might result in *N* single target amplification. In case of a doubt, it is recommended referring to a reference laboratory for further testing.
- Some samples (in *SARS-CoV-2 (N1 + N2)* reaction tube) may fail to exhibit *RNase P* amplification curves due to low human cell numbers in the original clinical sample. A negative IC signal does not preclude the presence of SARS-CoV-2, Flu and/or RSV RNA in a clinical specimen.
- A positive test result does not necessarily indicate the presence of viable viruses and does not imply that these viruses are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of targets viral sequences.
- Negative results do not preclude SARS-CoV-2, Flu and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 and novel Influenza A strain have not been determined. The collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- If diagnostic tests for other respiratory illnesses are negative and the patient's clinical presentation and epidemiological information suggest that SARS-CoV-2, Flu and/or RSV infection is possible, then a false negative result should be considered, and a re-testing of the patient should be discussed.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System retesting will be required. Unresolved results may be due to the presence of inhibitors in the sample or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System contains an internal control in each *Flu A, Flu B & RSV* reaction tube and an endogenous internal control in each *SARS-CoV-2 (N1 + N2)* reaction tube which confirms the correct performance of the technique.

12. Performance characteristics

12.1. Clinical sensitivity and specificity

The clinical performance of VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System was tested individually in each reaction tube.

The clinical performance of *Flu A, Flu B & RSV* reaction tube was tested using 344 respiratory specimens (oropharyngeal swabs) from symptomatic patients. These results were compared with those obtained with a molecular detection method (cobas® Influenza A/B & RSV (Roche)).

The results were as follows:

***Flu A, B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	157	2*	159
	-	7*	178	185
	Total	164	180	344

Table 10. Comparative results for Flu A.

Positive percent agreement is >96% and negative percent agreement is >99%.

**The low amount of template RNA in this respiratory sample is below the detection limit of the method used.*

***Flu A, Flu B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	99	4*	103
	-	1*	240	241
	Total	100	244	344

Table 11. Comparative results for Flu B.

Positive percent agreement is >99% and negative percent agreement is >98%.

**The low amount of template RNA in this respiratory sample is below the detection limit of the method used.*

***Flu A, Flu B & RSV* reaction tube**	**cobas® Influenza A/B & RSV (Roche)**			
		+	**-**	**Total**
	+	22	4*	26
	-	3*	315	318
	Total	25	319	344

Table 12. Comparative results for RSV.

Positive percent agreement is >88% and negative percent agreement is >99%.

*The low amount of template RNA in this respiratory sample is below the detection limit of the method used.

The clinical performance of *SARS-CoV-2 (N1 + N2)* reaction tube was tested using 254 respiratory samples (nasopharyngeal swabs in Vircell Transport medium) from patients with clinical suspicion of COVID-19 disease or other similar respiratory diseases. The results were compared with those obtained with the clinical diagnosis performed with Simplexa™ COVID-19 Direct assay with discrepant analysis performed with the Charité protocol.

SARS-CoV-2 (N1 + N2) reaction tube	Alternative RT-PCR assays			
		+	-	Total
	+	63	2*	65
	-	0	189	189
	Total	63	191	254

Table 13. Comparative results for SARS-CoV-2.

*Initial diagnose of one of the two samples was invalid and reported to the patient as positive for prevention and quarantine period.

SARS-CoV-2 (N1 + N2) reaction tube detected two positive samples that were not detected using Simplexa™ COVID-19 Direct assay and the Charité protocol.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) for *SARS-CoV-2 (N1 + N2)* reaction tube are >99% and 98%, respectively.

Results show high agreement to detect SARS-CoV-2, Flu A, Flu B and/or RSV viruses using VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

12.2. Analytical sensitivity

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System has a detection limit of ≥ 10 genome copies per reaction for Flu A, ≥ 20 genome copies per reaction for Flu B, ≥ 2 genome copies per reaction for RSV and ≥ 5 genome copies per reaction for SARS-CoV-2 with a positive rate of ≥ 95% (Figures 2, 3, 4, 5 and 6).

Figure 2. Dilution series of Flu A ($2*10^6$-$2*10^1$ copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

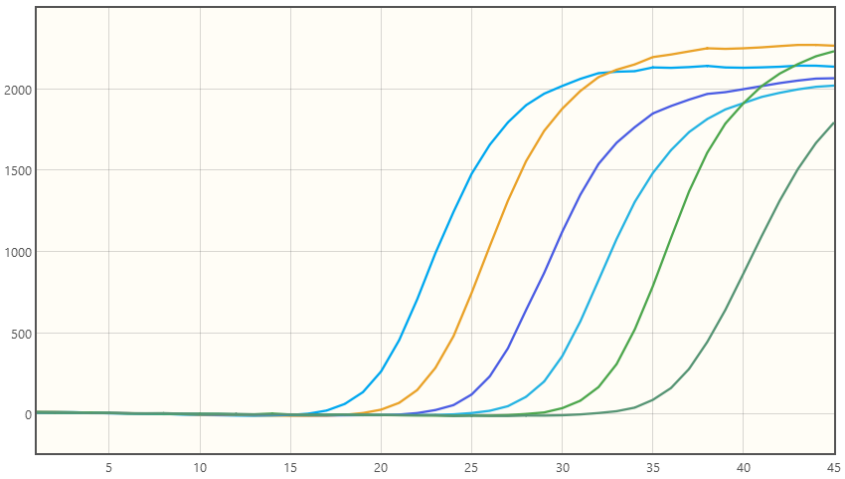


Figure 3. Dilution series of Flu B (2*10⁶-2*10¹ copies per reaction) template run on the BD MAX™ System (585/630 (ROX) channel).

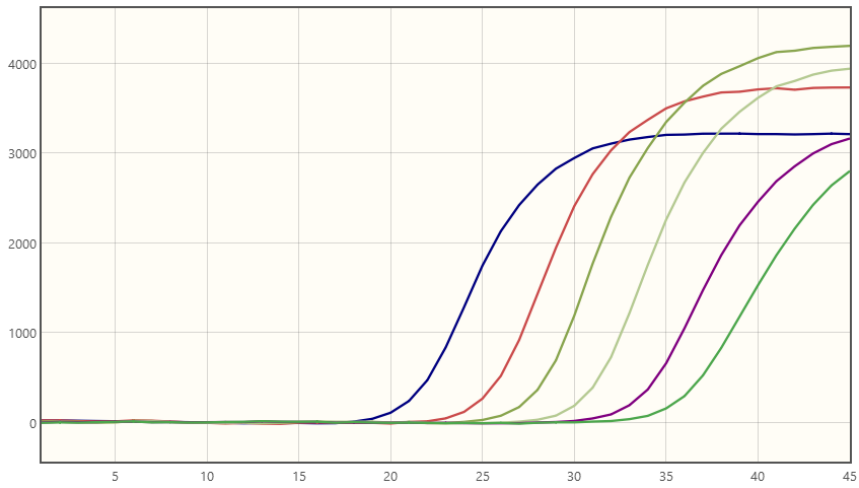


Figure 4. Dilution series of RSV (2*10⁶-2*10¹ copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).

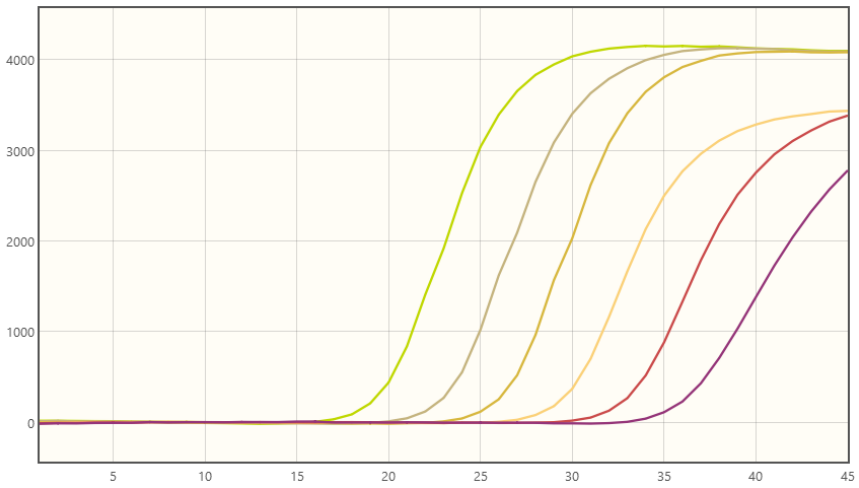


Figure 5. Dilution series of SARS-CoV-2 *(N1 + N2)* (9.9*10⁴-9.9*10⁰ and 5.0*10⁰ genome copies per reaction) template run on the BD MAX™ System (475/520 (FAM) channel).

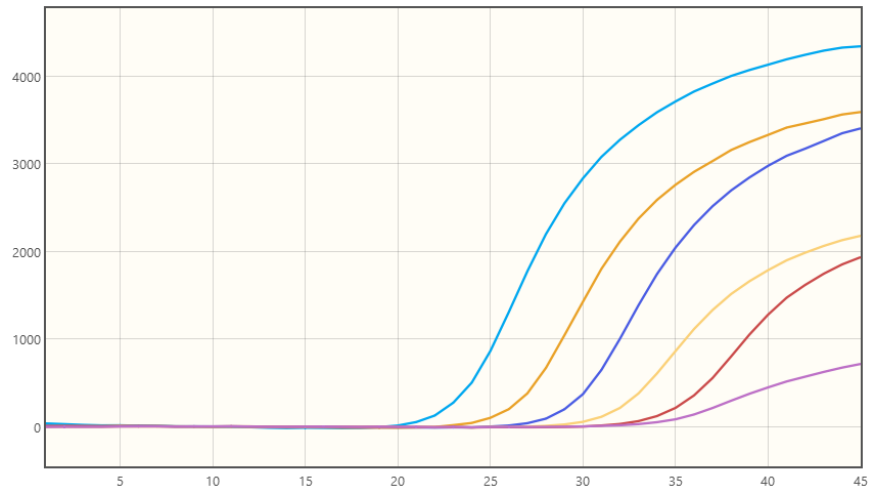
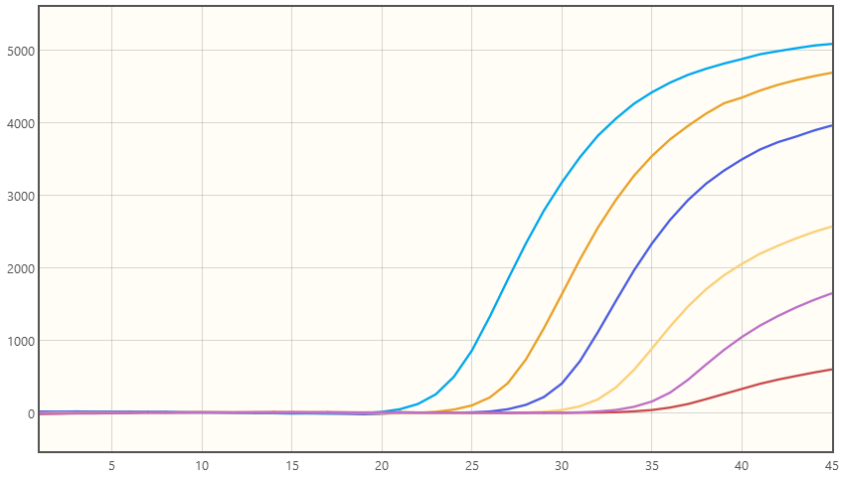


Figure 6. Dilution series of SARS-CoV-2 *(N1 + N2)* ($9.9*10^4$-$9.9*10^0$ and $5.0*10^0$ genome copies per reaction) template run on the BD MAX™ System (630/665 (Cy5) channel).



12.3. Analytical specificity

The specificity of the SARS-CoV-2, Flu (A+B) & RSV assay was confirmed by testing a panel consisting of different microorganisms representing the most common respiratory pathogens. No cross-reactivity was detected between any of the following microorganisms tested, except the targeted pathogens of each assay:

Cross-reactivity testing					
Human Adenovirus types 1-5, 8, 15, 31, 40 and 41	-	Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a)	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus	-/+
Bocavirus	-	Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a)	-/+	Influenza A/Chicken/Myanmar/433/2016 (H9N2) virus	-/+
Bordetella bronchiseptica	-	Influenza A/Newcastle/607/2019 (H3N2) virus	-/+	Influenza A/Hong Kong/1073/99 (H9N2) virus	-/+
Bordetella holmesii	-	Influenza A/New York/39/2012 (H3N2) virus	-/+	Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus	-/+
Bordetella parapertussis	-	Influenza A/Ohio/2/2012 (H3N2) virus	-/+	Influenza B/Brisbane/60/2008 virus	-/+
Bordetella pertussis	-	Influenza A/Perth/1001/2018 (H3N2) virus	-/+	Influenza B/Colorado/6/2017 virus	-/+
Chlamydia caviae	-	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus	-/+	Influenza B/Malaysia/2506/2004 virus	-/+
Chlamydia psittaci genotype A and C	-	Influenza A/South Australia/55/2014 (H3N2) virus	-/+	Influenza B/Maryland/15/2016 virus	-/+
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus	-/+	Influenza B/Netherlands/207/06 virus	-/+
Human coronavirus 229E, OC43, NL63 and HKU1	-	Influenza A/Switzerland/9715293/2013 (H3N2) virus	-/+	Influenza B/Netherlands/2518/2016 (clade 1A) virus	-/+

Cross-reactivity testing					
MERS Coronavirus	-	Influenza A/Texas/50/2012 (H3N2) virus	-/+	Influenza B/Nevada/3/2011 virus	-/+
SARS Coronavirus Strain Frankfurt 1	-	Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1)	-/+	Influenza B/New Jersey/1/2012 virus	-/+
SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 p.1	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus	-/+	Influenza B/Texas/02/2013 virus	-/+
SARS-CoV-2 strain 2019-nCoV/Italy-INMI1	-/+	Influenza A/Victoria/210/2009(H3N2) virus	-/+	Influenza B/Townsville/8/2016 virus	-/+
SARS-CoV-2 isolate Australia/VIC01/2020	-/+	Influenza A/Victoria/361/2011 (H3N2) virus	-/+	Influenza B/Canberra/11/2016 virus	-/+
SARS-CoV-2 isolate Wuhan-Hu-1	-/+	Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus	-/+	Influenza B/Florida/4/2006 virus	-/+
SARS-CoV-2 strain 2019nCoV/USAWA1/2020	-/+	Influenza A/Anhui/01/2005 (H5N1) virus	-/+	Influenza B/Florida/07/2004 virus	-/+
Enterovirus 68 and 71	-	Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus	-/+	Influenza B/Guangdong/120/2000 virus	-/+
Enterovirus Echovirus 11 and 30	-	Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus	-/+	Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus	-/+
Enterovirus Coxsackievirus A24, A9 and B3	-	Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus	-/+	Influenza B/ Jiangsu/10/2003 virus	-/+
Haemophilus influenzae MinnA	-	Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus	-/+	Influenza B/Massachusetts/2/2012 virus	-/+
Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus	-/+	Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus	-/+	Influenza B/Netherlands/365/2016 (clade 3) virus	-/+
Influenza A/California/7/2009(H1N1)pdm09 virus	-/+	Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus	-/+	Influenza A/duck/Hunan/795/2002 (H5N1) virus	-/+	Influenza B/Texas/06/2011 virus	-/+
Influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 virus	-/+
Influenza A/Michigan/45/2015 (H1N1)pdm09 virus	-/+	Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus	-/+	Influenza B/Wisconsin/1/2010 BX-41A virus	-/+
Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1)	-/+	Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus	-/+	*Legionella bozemanii*	-
Influenza A/New Caledonia/20/99(H1N1) virus	-/+	Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus	-/+	*Legionella dumoffii*	-
Influenza A/New York/18/2009 (H1N1)pdm09 virus	-/+	Influenza A/Hong Kong/213/2003 (H5N1) virus	-/+	*Legionella longbeachae*	-

Cross-reactivity testing					
Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09 virus	-/+	Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus	-/+	*Legionella micdadei*	-
Influenza A/Sydney/134/2018 (H1N1)pdm09 virus	-/+	Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus	-/+	*Legionella pneumophila*	-
Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus	-/+	Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus	-/+	Human metapneumovirus A and B	-
Influenza A/PR/8/34 (H1N1) virus	-/+	Influenza A/Vietnam/1194/2004 (H5N1) virus	-/+	*Moraxella catarrhalis*	-
Influenza A/Brisbane/117/2018 (H3N2) virus	-/+	Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus	-/+	*Mycoplasma pneumoniae*	-
Influenza A/Brisbane/1028/2017 (H3N2) virus	-/+	Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus	-/+	*Mycobacterium tuberculosis* not rifampin resistant	-
Influenza A/Fujian/411/2002 (H3N2) virus	-/+	Influenza A/Whooper Swan/R65/2006 (H5N1) virus	-/+	Human parainfluenza 1, 2, 3 and 4 viruses	-
Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus	-/+	*Pneumocystis jirovecii* Type A1 and g885652	-
Influenza A/Hong Kong/4801/2014 (H3N2) virus	-/+	Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus	-/+	Human rhinovirus type C	-
Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2) virus	-/+	Influenza A/Duck/Lao/XBY004/2014 (H5N6) (Clade 2.3.4.4) virus	-/+	*Staphylococcus aureus* subsp. *aureus*	-
Influenza A/Indiana/8/2011 (H3N2)v virus	-/+	Influenza A/DE-SH/Reiherente/AR8444/2013 (H5N8) virus	-/+	*Staphylococcus epidermidis*	-
Influenza A/Indiana/10/2011 (H3N2)v virus	-/+	Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus	-/+	*Streptococcus pneumoniae* Z022	-
Influenza A/Kansas/14/2017 (H3N2) virus	-/+	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus	-/+	*Streptococcus pyogenes*	-
Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus	-/+	*Streptococcus salivarius*	-
Influenza A/Kumamoto/102/2002 (H3N2) virus	-/+	Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus	-/+	Respiratory syncytial virus (RSV) A and B (strain CH93(18)-18)	-/+
Influenza A/Minnesota/11/2010 (H3N2)v virus	-/+	Influenza A/Anhui/1/2013 (H7N9) virus	-/+	Human Respiratory Syncytial Virus strain Long	-/+
Influenza A/Minnesota/11/2010 X203 (H3N2)v virus	-/+	Influenza A/Guangdong/17SF003/2016 (H7N9) virus	-/+		

Table 14. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **SARS-CoV-2** was evaluated against RNA from Human 2019-nCoV strain BetaCoV/Germany/BavPat1/2020 p.1,

Human 2019-nCoV strain 2019-nCoV/Italy-INMI1, SARS-CoV-2 strain 2019nCoV/USA-WA1/2020, synthetic RNA controls for two variants of the SARS-CoV-2 virus: MT007544.1 (SARS-CoV2 isolate Australia/VIC01/2020) and MN908947.3 (SARS-CoV-2 isolate Wuhan-Hu-1), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza A** was evaluated against RNA extracted from the following strains: Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09 virus, Influenza A/California/7/2009(H1N1)pdm09 virus, Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09 virus, Influenza A/Massachusetts/15/2013 (H1N1)pdm09 virus, Influenza A/Michigan/45/2015 (H1N1)pdm09 virus, Influenza A/Netherlands/1250/2016 (H1N1)pdm09 virus (clade 6B.1), Influenza A/New Caledonia/20/99(H1N1) virus, Influenza A/New York/18/2009 (H1N1)pdm09 virus, Influenza A/Singapore/GP1908/2015 virus, IVR-180 (H1N1)pdm09 virus, Influenza A/Sydney/134/2018 (H1N1)pdm09 virus, Influenza A/Victoria/2040/2018 (H1N1)pdm09 virus, Influenza A/PR/8/34 (H1N1) virus, Influenza A/Brisbane/117/2018 (H3N2) virus, Influenza A/Brisbane/1028/2017 (H3N2) virus, Influenza A/Fujian/411/2002 (H3N2) virus, Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2) virus, Influenza A/Hong Kong/4801/2014 (H3N2) virus, Influenza A/Hong Kong/4801/2014 NYMC X-263B (H3N2) virus, Influenza A/Indiana/8/2011 (H3N2)v virus, Influenza A/Indiana/10/2011 (H3N2)v virus, Influenza A/Kansas/14/2017 (H3N2) virus, Influenza A/Kansas/14/2017, NYMC X-327 (H3N2) virus, Influenza A/Kumamoto/102/2002 (H3N2) virus, Influenza A/Minnesota/11/2010 (H3N2)v virus, Influenza A/Minnesota/11/2010 X203 (H3N2)v virus, Influenza A/Netherlands/398/2014 (H3N2) virus (clade 3C.3a), Influenza A/Netherlands/2393/2015 (H3N2) virus (clade 3C.2a), Influenza A/Newcastle/607/2019 (H3N2) virus, Influenza A/New York/39/2012 (H3N2) virus, Influenza A/Ohio/2/2012 (H3N2) virus, Influenza A/Perth/1001/2018 (H3N2) virus, Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2) virus, Influenza A/South Australia/55/2014 (H3N2) virus, Influenza A/South Australia/55/2014, IVR-175 (H3N2) virus, Influenza A/Switzerland/9715293/2013 (H3N2) virus, Influenza A/Texas/50/2012 (H3N2) virus, Influenza A/Thüringen/5/2017 (H3N2) virus (Clade 3C2a.1), Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C) virus, Influenza A/Victoria/210/2009(H3N2) virus, Influenza A/Victoria/361/2011 (H3N2) virus, Influenza A/Victoria/361/2011 IVR-165 (H3N2) virus, Influenza A/Anhui/01/2005 (H5N1) virus, Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1) virus, Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus, Influenza A/chicken/Yunnan/1251/2003 (H5N1) virus, Influenza A/common magpie/Hong Kong/645/2006 (H5N1) virus, Influenza A/duck/Hunan/795/2002 (H5N1) virus, Influenza A/Egypt/321/2007 (H5N1) virus, Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1) virus, Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1) virus, Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 virus, Influenza A/Hong Kong/213/2003 (H5N1) virus, Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus, Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1) virus, Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1) virus, Influenza A/Vietnam/1194/2004 (H5N1) virus, Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1) virus, Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1) virus, Influenza A/Whooper Swan/R65/2006 (H5N1) virus, Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus, Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3) virus, Influenza A/Duck/Lao/XBY004/2014 (H5N6) virus (Clade 2.3.4.4), Influenza A/DE-SH/Reiherente/AR8444/2016 (H5N8) virus, Influenza A/Turkey/Germany/R2485-86/2014 (H5N8) virus, Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2) virus, Influenza A/Mallard/Netherlands/2/2009 (H7N7) virus, Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1 virus, Influenza A/Anhui/1/2013 (H7N9) virus, Influenza A/Guangdong/17SF003/2016 (H7N9) virus, Influenza A/Chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2) virus,

Influenza A/Chicken/Myanmar/433/2016 (H9N2) virus, Influenza A/Hong Kong/1073/99 (H9N2) virus, Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26 virus, showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza B** was evaluated against RNA extracted from the following strains: Influenza B/Brisbane/60/2008 virus, Influenza B/Colorado/6/2017 virus, Influenza B/Malaysia/2506/2004 virus, Influenza B/Maryland/15/2016 virus, Influenza B/Netherlands/207/06 virus, Influenza B/Netherlands/2518/2016 (clade 1A) virus, Influenza B/Nevada/3/2011 virus, Influenza B/New Jersey/1/2012 virus, Influenza B/Texas/02/2013 virus , Influenza B/Townsville/8/2016 virus (**B/Victoria lineage**); Influenza B/Canberra/11/2016 virus, Influenza B/Florida/4/2006 virus, Influenza B/Florida/07/2004 virus, Influenza B/Guangdong/120/2000 virus, Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39) virus, Influenza B/Jiangsu/10/2003 virus, Influenza B/Massachusetts/2/2012 virus, Influenza B/Netherlands/365/2016 (clade 3) virus, Influenza B/Phuket/3073/2013 virus, Influenza B/Texas/06/2011 virus, Influenza B/Wisconsin/1/2010 virus, Influenza B/Wisconsin/1/2010 BX-41A virus (**B/Yamagata lineage**), showing positive result.

The reactivity of the VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **RSV** was confirmed against RNA extracted from RSV A and B (strain CH93(18)-18) and Human Respiratory Syncytial Virus strain Long, showing positive result.

DANSK

1. Anvendelsesformål

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System er en automatiseret RT-PCR-test i realtid designet til kvalitativ påvisning og differentiering af RNA fra SARS-CoV-2, Influenza A, Influenza B og/eller Human respiratorisk syncytialvirus A/B (RSV) i luftvejsprøver fra personer, der er mistænkt for at have COVID-19 eller anden luftvejsinfektion af deres sundhedspersonale. Denne test er beregnet til at blive brugt som et hjælpemiddel til identifikation af tilstedeværelsen af SARS-CoV-2, Influenza A, Influenza B og/eller RSV viral RNA. Analysen anvender BD MAX™ System til automatisk ekstraktion af RNA og efterfølgende realtids RT-PCR med anvendelse af de medfølgende reagenser kombineret med universelle reagenser og engangsartikler til BD MAX™ System. RNA ekstraheres fra luftvejsprøver, forstærkes ved hjælp af RT-PCR og detekteres ved hjælp af fluorescerende reporterfarvesonder, der er specifikke for SARS-CoV-2, Influenza A, Influenza B og/eller RSV.

2. Oversigt og forklaring

Coronavirus er indkapslede ikke-segmenterede positive RNA-vira og tilhører *Coronaviridae*-familien. Seks coronavirusarter vides at forårsage sygdomme hos mennesker. Fire vira (229E, OC43, NL63 og HKU1) forårsager almindelige forkølelsessymptomer, og de to andre (svært akut respiratorisk syndrom coronavirus (SARS-CoV) og Mellemøstens respiratoriske syndrom coronavirus (MERS-CoV)) er zoonotiske og forårsager mere alvorlige komplikationer. SARS-CoV og MERS-CoV har forårsaget mere end 10.000 kumulative tilfælde i de seneste to årtier med en dødelighed på 34 % for MERS-CoV og for 10 % SARS-CoV.

I december 2019 havde nogle mennesker, der arbejdede på eller boede omkring Huanan skaldyrsmarked i Wuhan, Hubei-provinsen i Kina, lungebetændelse af ukendt årsag. Dyb sekvensanalyse af respirationsprøverne indikerede en ny coronavirus, som først fik navnet 2019 novel coronavirus (2019-nCoV) og for nylig SARS-CoV-2.

Overførsel af SARS-CoV-2 fra menneske til menneske er blevet bekræftet, selv i inkubationsperioden uden symptomer, og virusset forårsager alvorlige luftvejslidelser, som ligner dem SARS-CoV frembragte. Selv om lungebetændelse er den hyppigste sygdom, har enkelte patienter udviklet svær lungebetændelse, lungeødem, akut åndedrætsbesvær eller multiorgansvigt og død. Centers of Disease Control and Prevention (CDC) mener, at symptomer på SARS-CoV-2 kan opstå så få som 2 dage eller så længe som 14 dage efter eksponering, hvor de mest almindelige er feber eller kulderystelser, hoste, træthed, appetitløshed, myalgi og dyspnø. Mindre almindelige symptomer er ondt i halsen, tilstoppet næse hovedpine, diarré, kvalme og opkastning. Tab af lugt (anosmi) eller tab af smag (ageusi) forud for forekomsten af luftvejssymptomer er også blevet rapporteret. Ældre voksne og personer, der har alvorlige underliggende medicinske tilstande som hjerte- eller lungesygdom eller diabetes, synes at have større risiko for at udvikle mere alvorlige komplikationer fra COVID-19-sygdommen.

CDC anbefaler prøver fra de øvre luftveje (nasofaryngeale (NP) og orofaryngeale (OP) podepinde, nasal midt-turbinat-podepind, nasal podepinde, nasofaryngeal skylning/aspirat eller nasalskylning/aspirat (NW), der primært indsamles af sundhedspersonale) og/eller prøver fra de nedre luftveje (sputum, endotrakealt aspirat eller bronchoalveolær skylning hos patienter med mere alvorlig luftvejssygdom) til identifikation af SARS-CoV-2 og andre luftvejsvira, såsom influenza og RSV.

Influenzavirus tilhører familien *Orthomyxoviridae* og forårsager størstedelen af de nedre luftvejsinfektioner. Influenza A og B er en væsentlig årsag til sygelighed og dødelighed på verdensplan i betragtning af, at ældre og svækkede personer er særligt udsatte for at udvikle alvorlig sygdom og komplikationer såsom lungebetændelse. De fleste mennesker oplever nogle eller alle disse symptomer: feber eller feber/kulderystelser, hoste, ondt i halsen, tilstoppet næse og næseflåd, muskelsmerter, hovedpine og appetitløshed. Influenzavirusserne kan spredes fra person til person på to forskellige måder: gennem luften (store dråber og aerosoler fra nysen og hoste) og ved direkte eller indirekte kontakt.

Influenza A og B er et omsluttet, enkeltstrenget RNA-virus, der indeholder otte segmenterede strenge af genom-RNA, som typisk koder for 11 eller 12 virale proteiner. Viruskonvolutten, der stammer fra værtsplasmamembranen, består af en lipid-dobbeltlag indeholdende transmembranproteiner, som hæmagglutinin (HA) og neuraminidase (NA) og matrixproteiner M1 og M2. Influenza A-virus er yderligere klassificeret i undertyper baseret på antigeniciteten af deres "HA" og "NA" molekyler, mens Influenza B er opdelt i 2 antigenetisk og genetisk forskellige slægter, Victoria og Yamagata.

Human respiratorisk syncytialvirus A og B (RSV) tilhører *familien Paramyxoviridae* og er de vigtigste virale agenser ved akutte luftvejsinfektioner. RSV er et omsluttet, ikke-segmenteret, negativt, enkeltstrenget lineært RNA-genomvirus. Respiratorisk syncytialvirus er en almindelig bidragyder til luftvejsinfektioner og forårsager bronkitis, lungebetændelse, og kroniske obstruktive lungeinfektioner hos mennesker i alle aldre. Personerne oplever ofte nogle eller alle af disse symptomer: snue, lav grad af feber, hoste, ondt i halsen, hovedpine, og en hvæsende vejrtrækning. RSV overføres via store nasofaryngeale sekretionsdråber fra inficerede personer, tæt kontakt eller selvinokulering efter berøring af kontaminerede overflader.

Diagnose kan være problematisk, da en lang række patogener kan forårsage akutte luftvejsinfektioner med lignende kliniske symptomer. Realtids-PCR-analyser har vist sig at være et følsomme og specifikke diagnostiske værktøjer til påvisning af SARS-CoV-2-, influenza-A-, influenza-B- og RSV-vira.

3. Procedurens princip

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System er designet til identifikation af SARS-CoV-2, influenza A, influenza B og/eller RSV i luftvejsprøver. Detektionen foretages i et-trins realtids RT-PCR-format, hvor den omvendte transskription og den efterfølgende forstærkning af den specifikke målsekvens finder sted i samme reaktionsrør. Det isolerede RNA-mål transskriberes og genererer komplementært DNA ved revers transskriptase, som efterfølges af amplificering af to bevarede områder af *N-genet* (N1 og N2) for SARS-CoV-2, et bevaret område af *M1-genet* for influenza A og influenza B, og et bevaret område af *N-genet* for RSV ved hjælp af specifikke primere og fluorescensmærkede sonder.

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System er baseret på 5´exonukleaseaktivitet fra DNA-polymerase. Under DNA-forstærkningen spalter dette enzym proben, som er bundet til den komplementære DNA-sekvens og adskiller quencher-farvestoffet fra rapportøren. Denne reaktion genererer en stigning i det fluorescerende signal, som er proportional med mængden på målskabelonen. Denne fluorescens måles af BD MAX™-systemet.

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System består af to forskellige reaktionsrør. Et af rørene detekterer og skelner RNA fra influenza A, influenza B og/eller RSV (Transparent Red eller 1A folie), og det andet rør detekterer specifikt RNA fra SARS-CoV-2 (Transparent Grøn eller 1G-folie). Hvert rør indeholder alle de komponenter, der er nødvendige for realtids-PCR-analyse (specifikke primere/sonder, dNTPS, buffer, polymerase, revers-transkriptase) i et stabiliseret format samt en intern kontrol (endogen i SARS-CoV-2 reaction tube) til overvågning af ekstraktionsprocessen og/eller hæmning af polymeraseaktiviteten. SARS-CoV-2-analysen anvender et humant husholdningsgen som en endogen intern kontrol (humant *RNase P-gen*). Humane husholdningsgener er involveret i grundlæggende cellevedligeholdelse og forventes derfor at være til stede i alle nukleerede humane celler og opretholde relativt konstante ekspressionsniveauer. Hvert RNA-mål forstærkes og detekteres i specifikke kanaler (475/520, 585/630, og/eller 630/665) og den interne kontrol (IC) i kanal 530/565. I influenza A-, influenza B- og/eller RSV-analysen forstærkes og detekteres influenza A-RNA-målet i kanal 475/520, influenza B-RNA-målet i kanal 585/630, RSV-RNA-målet i kanal 630/665 og den interne kontrol (IC) af denne analyse i kanal 530/565. I SARS-CoV-2-analysen forstærkes og detekteres N2-målet i kanal 475/520, N1-målet i kanal 630/665 og den endogene interne kontrol (IC) i kanal 530/565.

4. Leverede reagenser

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System indeholder følgende materialer og reagenser, der er beskrevet i Tabel 1:

Reference	Reagens/Materiale	Beskrivelse	Farve/stregkode	Mængde
VS-ABR212R	*Flu A, Flu B & RSV* reaction tube	En blanding af enzymer, primerprober, buffere, dNTP'er, stabilisatorer og interne kontroller i stabiliseret format	Transparent Rød eller 1A-folie	2 poser med 12 rør
VS-NCO312	*SARS-CoV-2 (N1 + N2)* reaction tube	En blanding af enzymer, primerprober, buffere, dNTP'er, stabilisatorer og endogene interne kontroller i stabiliseret format	Transparent Grøn eller 1G folie	2 poser med 12 rør
VS-RB09	Rehydration Buffer tube	Opløsning til rekonstitution af det stabiliserede produkt	Transparent Orange eller 11 folie	1 pose med 24 rør

Tabel 1. Reagenser og materialer i VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System med kat. nr. VS-FNR124 (444217).

5. Reagenser og udstyr, der skal leveres af brugeren

Følgende liste omfatter materialer og udstyr, der er nødvendige til brug, men som ikke er inkluderet i VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

- Realtids-PCR-instrument: BD MAX™ System.
- BD MAX™ ExK™ TNA-3 (Ref:442827 eller 442828)
- BD MAX™ PCR Cartridges (Ref: 437519)
- vortex.
- Mikropipetter (nøjagtighed mellem 2 og 1000 µl).
- Filterspidser.

- Pulverfrie engangshandsker

6. Transport- og opbevaringsforhold

- Sættene kan sendes og opbevares ved 2 - 40 °C, indtil den udløbsdato, der er angivet på etiketten.
- Efter åbning af aluminiumsposerne, som indeholder reaktionsrørene, kan de anvendes i op til 28 dage.

7. Særlige forholdsregler for brugere

- Produktet er tiltænkt professionelle brugere, såsom laboratorie- eller sundhedspersonale og teknikere, der er uddannet i molekylærbiologiske teknikker.
- Til *in vitro-diagnostisk* brug.
- Brug ikke reagenser og/eller materialer, hvis udløbsdatoen er overskredet.
- Brug ikke sættet, hvis etiketten, der forsegler den ydre æske, er i stykker.
- Brug ikke reagenser, hvis beskyttelsesæsken er åben eller i stykker ved ankomsten.
- Brug ikke reagenser, hvis beskyttelsesposerne er åbne eller i stykker ved modtagelsen.
- Brug ikke reagenser, hvis tørremidlet ikke er til stede eller er i stykker inden i reagensposerne.
- Tørremidlet må ikke fjernes fra reagensposerne.
- Luk straks de beskyttende poser med reagenser med lynlåsforseglingen efter hver brug. Fjern eventuel overskydende luft i poserne inden forsegling.
- Brug ikke reagenser, hvis folien er blevet ødelagt eller beskadiget.
- Reagenser fra forskellige poser og/eller sæt og/eller partier må ikke blandes.
- Beskyt reagenser mod fugt. Længerevarende eksponering for fugt kan påvirke produktets ydeevne.
- Hold komponenterne væk fra lys.
- I tilfælde, hvor andre PCR-test udføres i det samme generelle område af laboratoriet, skal det sikres, at VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System, BD MAX™ ExK™ TNA-3-ekstraktionssættet, eventuelle yderligere reagenser, der er nødvendige for testen, og BD MAX™ System ikke er kontamineret. Undgå altid mikrobiel kontaminering og kontaminering af reagenser med ribonuklease (RNase)/deoxyribonuklease (DNase). Det anbefales at anvende sterile RNase/DNase-fri aerosolresistente engangspipettespidser eller positive fortrængningspipettespidser. Brug en ny spids til hver prøve. Handsker skal udskiftes før håndtering af reagenser og kassetter.
- Sørg for at bruge et rør til at bestemme RNA fra Influenza A, Influenza B og RSV i Snap-In 2 (grøn position) og et andet rør til at bestemme RNA fra SARS-CoV-2 i Snap-In 4 (blå position). Pas på ikke at blande dem under hele processen.
- For at undgå kontaminering af miljøet med amplikoner må BD MAX™ PCR Cartridge ikke brydes fra hinanden efter brug. Forseglingerne på BD MAX™ PCR Cartridge er designet til at forhindre kontaminering.
- Tilrettelæg en ensrettet arbejdsgang. Den skal begynde i ekstraktionsområdet og derefter flyttes til forstærknings- og detektionsområdet. Prøver, udstyr og reagenser må ikke returneres til det område, hvor det foregående trin blev udført.
- Følg god laboratoriepraksis. Brug beskyttelsestøj, engangshandsker, beskyttelsesbriller og maske. Man må ikke spise, drikke eller ryge i arbejdsområdet. Vask hænder efter endt test.

- Prøverne skal behandles som potentielt smitsomme samt alle reagenser og materialer, der er blevet eksponeret for prøverne, og skal håndteres i overensstemmelse med de nationale sikkerhedsforskrifter. Træf de nødvendige forholdsregler under indsamling, opbevaring, behandling og bortskaffelse af prøver.
- Regelmæssig dekontaminering af almindeligt anvendt udstyr anbefales, især mikropipetter og arbejdsflader.
- Se brugervejledningen til BD MAX™-systemet for yderligere advarsler, forholdsregler og procedurer.

8. Procedure

8.1. INDSAMLING, OPBEVARING OG TRANSPORT AF PRØVER

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System er blevet valideret på nasofaryngeal/orofaryngeal podepind opsamlet i virale transportmedier (VTM) Vircell S.L., Spanien).

Andre typer prøver fra nasofaryngeal/orofaryngeale podepinde i VTM skal valideres af brugeren.

Prøveudtagning, opbevaring og transport skal vedligeholdes i overensstemmelse med de betingelser, der er valideret af brugeren. Samlet set skal luftvejsprøver indsamles og mærkes på passende vis i rene beholdere med eller uden transportmidler (afhængigt af prøvetype) og behandles så hurtigt som muligt for at garantere testens kvalitet. Prøverne skal transporteres ved 2 til 8 °C i op til 48 timer i henhold til lokale og nationale bestemmelser for transport af patogent materiale. Ved langtidstransport (mere end 48 timer) anbefaler vi forsendelse ved ≤-20 °C. Det anbefales at anvende friske prøver til testen. Prøverne kan opbevares ved 2 til 8 °C i op til 48 timer eller nedfryses ved -20 °C eller ideelt ved -70 °C for konservering. Gentagne fryse-tø-cyklusser bør undgås for at forhindre nedbrydning af prøven og nukleinsyrer.

8.2. KLARGØRING AF PRØVER OG RNA-EKSTRAKTION

Udfør prøveforberedelsen i overensstemmelse med anbefalingerne i brugsanvisningen til det anvendte ekstraktionssæt, BD MAX™ ExK™ TNA-3. Bemærk, at nogle andre prøver kan kræve forbehandling. Brugeren skal udvikle og validere ekstraktions- og præparationsprocedurer, der er specifikke til formålet.

1. Der pipetteres 400 µl nasofaryngeale/orofaryngeale podepindsprøver opsamlet i virale transportmedier (VTM) i et BD MAX™ TNA-3 Sample Buffer Tube, og røret lukkes med en septumhætte. Der sikres fuldstændig blanding ved at hvirvle prøven ved høj hastighed i 1 minut. Fortsæt til BD MAX™ Systembetjening.

Bemærk: *Flu A, Flu B & RSV* reaction tube er blevet valideret med en prøvevolumen på 200-400 µl og *SARS-CoV-2 (N1 + N2)* reaction tube med en prøvevolumen på 400-750 µl.

8.3. PCR-PROTOKOL

Bemærk: Der henvises til brugervejledningen til BD MAX™-systemet for at få detaljerede instruktioner.

8.3.1. Oprettelse af PCR-testprogram til VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System

Bemærk: Hvis du allerede har oprettet VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection test, kan du springe trin 8.3.1 over og gå direkte til 8.3.2.

1) Vælg fanen "Test Editor" (Testredigering) på skærmen "Run" (Kør) på BD MAX™-systemet.
2) Klik på knappen "Create" (Opret).
3) Navngiv din test i fanen Basic Information (Grundlæggende oplysninger) i vinduet "Test Name" (Testnavn): dvs. VIASURE *SARS-CoV-2, Flu (A+B) & RSV* (VSARSCoV2, FluA+B,RSV).
4) I rullemenuen "Extraction Type" (Ekstraktionstype), vælg "ExK TNA-3".
5) I rullemenuen "Master Mix Format" skal du vælge "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) I "Sample extraction parameters" (Parametre for prøveekstraktion) vælges "User defined" (Brugerdefineret), og prøvevolumen justeres til 950 µl.
7) I "Ct Calculation" (Ct-beregning) vælges "Call Ct at Threshold Crossing" (Beregn Ct når tærsklen krydses).
8) Hvis du kører softwareversion 5.00 eller nyere og har snap-in-rør med stregkodet folie, skal du vælge følgende konfiguration i "Custom Barcodes" (Brugerdefinerede stregkoder):
    a. Snap-In 2-stregkode: 1A (vedrørende *Flu A, Flu B & RSV* reaction tube)
    b. Snap-In 3 stregkode: 11 (vedrørende Rehydration Buffer tube)
    c. Snap-In 4 stregkode: 1G (vedrørende *SARS-CoV-2 (N1 + N2)* reaction tube)
9) "PCR Settings" (PCR-indstillinger) og "Test Steps" (Testtrin) skal udføres for Snap-In 2 (grøn) og Snap-In 4 (blå) positioner.
10) Snap-In 2 (grøn). Indtast følgende parametre på fanen "PCR settings" (PCR-indstillinger): "Channel Settings" (Kanalindstillinger), "Gains" (Stigninger) og "Threshold" (Tærskel) (Tabel 2).

Channel (Kanal)	Alias (Alias)	Gain (Gevinst)	Threshold (Tærskel)	Ct Min (Ct Min)	Ct Max (Ct Max)
475/520 (FAM)	Influenza A	60	100	0	40
530/565 (HEX)	IC	80	300	0	40
585/630 (ROX)	Influenza B	60	200	0	40
630/665 (Cy5)	RSV	60	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Tabel 2. PCR-indstillinger.

Bemærk: Det anbefales som udgangspunkt at fastsætte ovennævnte minimumstærskelværdier for hver kanal, men de endelige indstillinger skal fastlægges af slutbrugeren under resultatfortolkningen for at sikre, at tærskelværdierne falder inden for fluorescenskurvernes eksponentielle fase og over ethvert baggrundssignal. Tærskelværdien for forskellige instrumenter kan variere på grund af forskellige signalintensiteter.

11) Snap-In 2 (grøn). I fanen "PCR settings" (PCR-indstillinger) indtastes følgende parametre samt "Spectral Cross Talk" (Spektral krydstale) (tabel 3).

		False Receiving Channel (Falsk modtagekanal)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Excitationskanal)	475/520	-	0,0	0,0	0,0	0,0
	530/565	0,0	-	2,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	4,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tabel 3. Parametre for spektral krydstale.

12) Snap-In 2 (grøn). Indtast PCR-protokollen (tabel 4) på fanen "Test Steps" (Testtrin).

Step Name (Trinnavn)	**Profile Type (Profiltype)**	**Cycles (Cyklusser)**	**Time (s) (Tid (er))**	**Temperature (Temperatur)**	**Detect (Registrering)**
Reverse transcription (Revers transskription)	Hold	1	900	45 °C	-
Initial denaturation (Indledende denaturering)	Hold	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection) (Denaturering og annotering/udvidelse (Dataindsamling))	2-temperatur	45	10	95 °C	-
			61,1	63 °C	✓

Tabel 4. PCR-protokol.

13) Snap-In 4 (blå). Indtast følgende parametre på fanen "PCR settings" (PCR-indstillinger): "Channel Settings" (Kanalindstillinger), "Gains" (Stigninger) og "Threshold" (Tærskel) (Tabel 5).

Channel (Kanal)	**Alias (Alias)**	**Gain (Gevinst)**	**Threshold (Tærskel)**	**Ct Min (Ct Min)**	**Ct Max (Ct Max)**
475/520 (FAM)	SARS-CoV-2 N2-mål	80	150	0	40
530/565 (HEX)	Endogen IC	80	150	0	35
585/630 (ROX)	-	0	0	0	0
630/665 (Cy5)	SARS-CoV-2 N1-mål	80	150	0	40
680/715 (Cy5.5)	-	0	0	0	0

Tabel 5. PCR-indstillinger.

Bemærk: Det anbefales som udgangspunkt at fastsætte ovennævnte minimumstærskelværdier for hver kanal, men de endelige indstillinger skal fastlægges af slutbrugeren under resultatfortolkningen for at sikre, at tærskelværdierne falder inden for fluorescenskurvernes eksponentielle fase og over ethvert baggrundssignal. Tærskelværdien for forskellige instrumenter kan variere på grund af forskellige signalintensiteter.

14) Snap-In 4 (blå). I fanen "PCR settings" (PCR-indstillinger) indtastes følgende parametre samt "Spectral Cross Talk" (Spektral krydstale) (tabel 6).

		False Receiving Channel (Falsk modtagekanal)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Excitationskanal)	475/520	-	3,0	0,0	0,0	0,0
	530/565	1,0	-	0,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	0,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tabel 6. Parametre for spektral krydstale.

15) Snap-In 4 (blå). Indtast PCR-protokollen (tabel 7) på fanen "Test Steps" (Testtrin).

Step Name (Trinnavn)	Profile Type (Profiltype)	Cycles (Cyklusser)	Time (s) (Tid (er))	Temperature (Temperatur)	Detect (Registrering)
Reverse transcription (Omvendt transkription)	Hold	1	900	45 °C	-
Initial denaturation (Indledende denaturering)	Hold	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection) (Denaturering og annotering/udvidelse (dataindsamling))	2-temperatur	45	10	95 °C	-
			61,1	63 °C	✓

Tabel 7. PCR-protokol.

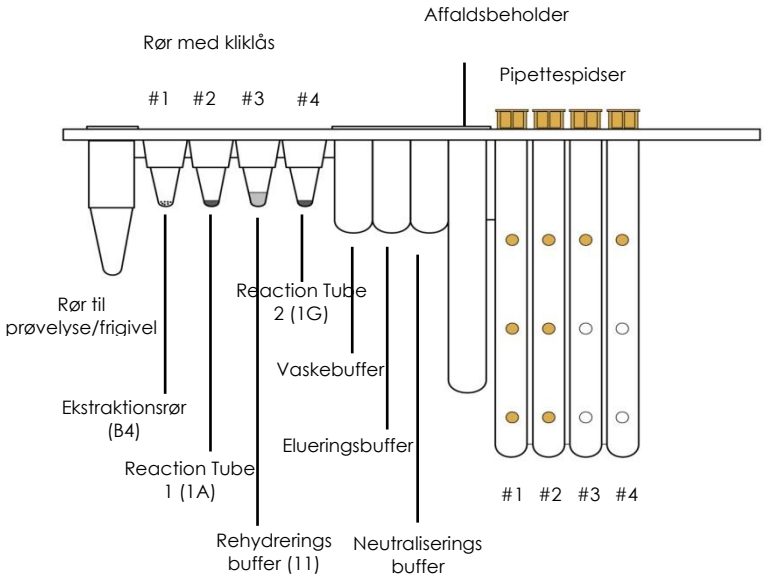
16) Klik på knappen "Save Test" (Gem test).

8.3.2. Opsætning af BD MAX™-stativ

1) For hver prøve, der skal testes, fjernes en Unitized Reagent-strimmel fra BD MAX™ ExK TNA-3 sættet. Bank forsigtigt hver strimmel mod en hård overflade for at sikre, at alle væskerne ligger i bunden af rørene, og anbring dem i BD MAX™-systemets prøvestativer.
2) Fjern det nødvendige antal BD MAX™ ExK™ TNA Extraction Tubes (B4) (hvid folie) fra deres beskyttelsespose. Sæt udtræksrøret(-rørene) (hvid folie) i de tilsvarende positioner i TNA-strimlen (fastgør position 1, hvid farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk posen med lynlåsforseglingen.
3) Bestem og separer det relevante antal rør af typen *Flu A, Flu B & RSV* reaction tube (rød eller 1A-folie) og fastgør dem til deres tilsvarende positioner i strimlen (fastgørelsesposition 2, grøn farvekodning på stativet. Se Figur 1).
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.

4) Fjern det nødvendige antal Rehydration Buffer tubes (orange eller 11 folie) og fastgør til deres tilsvarende positioner i strimlen (fastgørelsesposition 3, ikke-farvekodning på stativet. Se Figur 1). Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    a. For at sikre, at overførslen udføres korrekt, skal man sørge for, at væsken ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
5) Bestem og separer det relevante antal rør af typen SARS-CoV-2 (N1 + N2) reaction tube (grøn eller 1G folie) og fastgør til deres tilsvarende positioner i strimlen (snap position 4, blå farvekodning på stativet. Se Figur 1).
    a. Fjern overskydende luft, og luk aluminiumsposerne med lynlåsforseglingen.
    b. Rehydreringen udføres korrekt ved at sørge for, at det frysetørrede produkt ligger i bunden af røret og ikke er i kontakt med rørets top eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.

Figur 1. BD MAX™ TNA reagensstrimmel (TNA) fra BD MAX™ ExK TNA-3 kit.



8.3.3. BD MAX™ Instrumentopsætning

1) Vælg fanen "Work List" (Arbejdsliste) på skærmen "Run" (Kør) på BD MAX™ Systemsoftware v4.50A eller nyere.
2) Vælg VSARSCoV2, FluA+B,RSV i rullemenuen "Test" (hvis det ikke allerede er oprettet, se afsnit 8.3.1).
3) Vælg det relevante lotnummer for kittet (fremgår af ekstraktionskittets udvendige æske) fra rullemenuen (valgfrit).
4) Indtast prøvebufferrørets identifikationsnummer i vinduet Sample tube (Prøverør) på Worklist (Arbejdsliste), enten ved at scanne stregkoden med scanneren eller ved manuel indtastning.
5) Udfyld vinduet Specimen/Patient ID og/eller Accession på Worklist (arbejdsliste), og klik på knappen "Save" (Gem). Fortsæt, indtil alle prøvebufferrør er indtastet. Sørg for, at prøve-/patient-id'et og prøvebufferrørene matcher nøjagtigt.
6) Anbring det klargjorte prøvebufferrør i BD MAX™-stativet/stativerne.

7) Sæt stativet/stativerne i BD MAX™-systemet (stativ A er placeret i venstre side af BD MAX™-systemet og stativ B i højre side).
8) Anbring det nødvendige antal BD MAX™ PCR Cartridges i BD MAX™-systemet.
9) Luk lågen til BD MAX™-systemet.
10) Klik på "Start Run" (Start procedure) for at starte proceduren.

8.3.4 BD MAX™ rapport

1) Klik på knappen "Results" (Resultater) i hovedmenuen.
2) Dobbeltklik enten på din kørsel på listen, eller tryk på knappen "View" (Vis).
3) Klik på "Print" (Udskriv), vælg: "Run Details, Test Details and Plot…" (Kør detaljer, testdetaljer og tegn grafik)
4) Klik på knappen "Print or Export" (Udskriv eller eksportér) på skærmbilledet Run Reports (Kør rapporter).

9. Tolkning af resultater

For en detaljeret beskrivelse af, hvordan man analyserer data, se BD MAX™-systemets brugervejledning.

Analysen af data udføres som BD MAX™-software i overensstemmelse med producentens anvisninger. BD MAX™-softwaren rapporterer Ct-værdier og stigningskurver for hver detektorkanal for hver prøve, og testes på følgende måde:

- En Ct-værdi på 0 angiver, at der ikke blev beregnet nogen Ct-værdi af softwaren ved den angivne tærskelværdi (se tabel 2). En forstærkningskurve for prøven, der viser en Ct-værdi på "0", skal kontrolleres manuelt.

- Ct-værdien -1 angiver, at ingen forstærkningskurve er forekommet.

- Enhver anden Ct-værdi skal fortolkes i sammenhæng med forstærkningskurve og i overensstemmelse med retningslinjerne for tolkning af prøven som anført i Tabel 8 og 9.

Kontrollér, at det indvendige styresignal fungerer korrekt for amplifikationsblandingen. Desuden skal du kontrollere, at der ikke foreligger nogen rapport over BD MAX™ Systemfejl.

Resultaterne skal læses og analyseres ved hjælp af følgende tabeller:

a. *Flu A, Flu B & RSV* reaction tube: Snap-In 2

Flu A (475/520)	Flu B (585/630)	RSV (630/665)	Intern kontrol (530/565)	Fortolkning
+	+	+	+/-[1]	**Influenza A, influenza B og RSV RNA påvist[1]**
+	-	-	+/-[1]	**Influenza A RNA påvist, influenza B og RSV RNA ikke påvist[1]**
+	+	-	+/-[1]	**Influenza A og influenza B RNA påvist og RSV RNA ikke påvist[1]**
+	-	+	+/-[1]	**Influenza A og RSV RNA påvist, og influenza B RNA ikke påvist[1]**
-	+	-	+/-[1]	**Influenza B RNA påvist, influenza A og RSV RNA ikke påvist[1]**
-	+	+	+/-[1]	**Influenza B og RSV RNA påvist, influenza A RNA ikke påvist[1]**
-	-	+	+/-[1]	**RSV RNA påvist, influenza A og influenza B RNA ikke påvist[1]**
-	-	-	+[2]	**Influenza A, influenza B og RSV RNA ikke påvist[2]**
-	-	-	-[2]	**Resultatet Unresolved (uløst) (UNR) optræder under tilstedeværelse af hæmmere i PCR-reaktionen eller når der opstår et overordnet problem (der ikke rapporteres med en fejlkode) under prøvekørslen og/eller forstærkningstrinnene.[2]**
IND	IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™-systemet. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes fejl i BD MAX™-systemet. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 8. Tolkning af prøveresultater for Flu A, Flu B & RSV reaction tube

+: Forstærkning forekom
-: Ingen forstærkning forekom

1 En prøve betragtes som positiv, hvis den indhentede Ct-værdi er mindre end 40. Den interne kontrol kan vise et forstærkningssignal, fordi et stort antal målkopier kan medføre præferentiel forstærkning af målspecifikke nukleinsyrer i stedet for den interne kontrol. I disse tilfælde er undersøgelse af IC ikke nødvendig.

2 En prøve betragtes som negativ, hvis prøven viser intet forstærkningssignal, men den interne kontrol er positiv (Ct mindre end 40). En hæmning af PCR-reaktionen kan udelukkes ved forstærkning af intern kontrol. I tilfælde af uopklarede resultater (UNR), fravær af internt kontrolsignal i negativ prøve anbefales det at gentage analysen.

b. *SARS-CoV-2 (N1 + N2)* reaction tube: Snap-In 4

SARS-CoV-2 (N2 target) (475/520)	Endogenous Internal Control (530/565)	SARS-CoV-2 (N1 target) (630/665)	Fortolkning
+	+/-[3]	+	**SARS-CoV-2 *N gen*-RNA påvist [3]**
+[4]	+/-[3]	-	**SARS-CoV-2 *N gen*-RNA påvist [3,4]**
-	+/-[3]	+[4]	**SARS-CoV-2 *N gen*-RNA påvist [3,4]**
-	+[5]	-	**SARS-CoV-2 *N* gen-RNA ikke påvist[5]**
-	-[5]	-	**Resultatet Unresolved (uløst) (UNR) optræder under tilstedeværelse af hæmmere i PCR-reaktionen eller når der opstår et overordnet problem (der ikke rapporteres med en fejlkode) under prøvekørslen og/eller forstærkningstrinnene.[5]**
IND	IND	IND	**Analyseresultatet er Indeterminate (ubestemmeligt) (IND). Skyldes en fejl i BD MAX™-systemet. Analyseresultat, der vises i tilfælde af en instrumentfejl, der knyttet til en fejlkode.**
INC	INC	INC	**Analyseresultatet er Incomplete (ufuldstændigt) (INC). Skyldes fejl i BD MAX™-systemet. Analyseresultatet vises, hvor en fuldstændig kørsel ikke kunne gennemføres.**

Tabel 9. *Prøvefortolkning SARS-CoV-2 (N1 + N2)* reaction tube

+: Der opstod forstærkning

-: Der opstod ingen forstærkning

3 En prøve betragtes som positiv, hvis Ct-værdien er mindre end 40. Den endogene interne kontrol (IC) kan både vise et forstærkersignal eller intet forstærkersignal. Sommetider er IC-detektionen ikke nødvendig, fordi et højt kopinummer for målet kan forårsage præferenceamplifikation af målspecifikke nukleinsyrer.

4 Hvis kun ét af *N-genets* målområder forstærkes, skal man kontrollere kurvens sigmoide form og fluorescensens intensitet. I tilfælde af en tvivlsom fortolkning anbefales det afhængigt af det tilgængelige materiale også at:

a) ekstrahere og teste endnu en delprøve af samme prøve (om muligt øges prøvevolumen til 750 µl), eller

b) udtage en ny prøve og foretage en ny prøvning.

5 Hvis SARS-CoV-2-målområdet er negativt, skal den interne kontrol (IC) vise et forstærkningssignal med Ct på mindre end 35. Ct-værdien kan være meget variabel på grund af den endogene interne kontrol er et humant rengøringsgen, der bør være til stede i alle humane kerneceller i den oprindelige prøve. Hvis der mangler et signal eller en Ct-værdi ≥ 35 i den endogene interne kontrol, betragtes resultatet som "uopklaret", og det er nødvendigt at teste igen.

I tilfælde af et fortsat tvetydigt resultat anbefales det at gennemgå brugsanvisningen, den ekstraktionsproces, som brugeren anvender; til at verificere den korrekte ydeevne for hvert RT-qPCR-trin og gennemgå parametrene og kontrollere kurvens sigmoide form og fluorescensintensiteten.

Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.

10. Begrænsninger i testen

- Resultaterne af testen bør vurderes af en læge på baggrund af anamnese, kliniske symptomer og andre diagnostiske tests.
- Selv om denne test kan bruges sammen med andre typer prøver er den blevet valideret med nasofaryngeale/orofaryngeale podepinde indsamlet i VTM.
- For god testydeevne skal det frysetørrede produkt være i bunden af røret og ikke klæbe til det øverste område af røret eller folieforseglingen. Bank forsigtigt hvert rør mod en hård overflade for at sikre, at alt produktet er i bunden af røret.
- Et udseende af reaktionsblandingen i stabiliseret format, som normalt findes i bunden af røret, forskelligt fra det sædvanlige (uden konisk form, inhomogent, mindre/større i størrelse og/eller farve forskellig fra hvidlig) ændrer ikke testens funktionalitet.
- Testens kvalitet afhænger af prøvens kvalitet; korrekt ekstraheret nukleinsyre fra luftvejsprøver skal ekstraheres.
- Denne test er en kvalitativ test og giver ikke kvantitative værdier eller angiver antallet af tilstedeværende organismer.
- Meget lave målniveauer under detektionsgrænsen kan påvises, men resultaterne er muligvis ikke reproducerbare.
- Der er mulighed for falsk positive resultater som følge af krydskontaminering med SARS-CoV-2, Flu A, Flu B og/eller RSV, enten prøver indeholdende høje koncentrationer af mål-RNA eller kontaminering som følge af PCR-produkter fra tidligere reaktioner.
- De specifikke primer- og sondekombinationer til påvisning af bevarede områder af *N gene* (SARS-CoV-2), der anvendes til VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System, er designet på grundlag af den amerikanske CDC-analyse til specifik påvisning af SARS-CoV-2 ved at forstærke to unikke områder af N-genet. De udviser ikke signifikante kombinerede homologier med det menneskelige genom, den menneskelige mikroflora, SARS-CoV eller andre coronavira, hvilket kan resultere i forudsigelige falsk-positive resultater.
- Falsk-negative resultater kan skyldes flere faktorer og kombinationer heraf, herunder:
    - Forkerte metoder til indsamling, transport, opbevaring og/eller håndtering af prøver.
    - Forkerte behandlingsprocedurer (herunder RNA-ekstraktion).
    - Nedbrydning af det virale RNA under forsendelse/opbevaring og/eller behandling af prøver.
    - Mutationer eller polymorfismer i primer- eller sondebindingsområder kan påvirke påvisning en af nye eller ukendte SARS-CoV-2, Flu og/eller RSV.
    - En virusmængde i prøven under detektionsgrænsen for analysen.
    - Tilstedeværelsen af RT-qPCR-hæmmere eller andre typer interfererende stoffer.
    - Manglende overholdelse af brugsanvisningen og analyseproceduren.
- I *SARS-CoV-2 (N1 + N2)* reaction tube tyder en enkelt målstedsforstækrning eller endda tilfældige positive resultater tyder på et let afvigende forstærkningsudbytte for målstedet på *N-genet*. Prøver med lav virusmængde kan resultere i en enkelt målstedsforstærkning på N-genet. I tvivlstilfælde anbefales det at henvise til et referencelaboratorium med henblik på yderligere testning.

- Nogle prøver (i *SARS-CoV-2 (N1 + N2)* reaction tube) vil i nogle tilfælde ikke udvise *RNase P*-forstærkningskurver på grund af lave humane celletal i den oprindelige kliniske prøve. Et negativt IC-signal udelukker ikke tilstedeværelsen af SARS-CoV-2, Flu og/eller RSV RNA i en klinisk prøve.
- Et positivt testresultat indikerer ikke nødvendigvis tilstedeværelsen af levedygtige vira og betyder ikke, at disse vira er smitsomme eller forårsager kliniske symptomer. Et positivt resultat indikerer imidlertid tilstedeværelsen af målvirussekvenser.
- Negative resultater udelukker ikke SARS-CoV-2, influenza og/eller RSV-infektion og bør ikke anvendes som eneste grundlag for behandling eller andre beslutninger om patientbehandling. Optimale prøvetyper og tidspunktet for maksimale virusniveauer under infektioner forårsaget af SARS-CoV-2 og novel Influenza A-stamme er ikke fastlagt. Det kan være nødvendigt at indsamle flere prøver (typer og tidspunkter) fra samme patient for at påvise virusset.
- Hvis diagnostiske test for andre luftvejssygdomme er negative, og patientens kliniske præsentation og epidemiologiske oplysninger antyder, at SARS-CoV-2, influenza-infektion og/eller RSV-infektion er mulig, bør et falsk negativt resultat overvejes, og en ny test af patienten bør drøftes.
- I tilfælde af at opnå uopklarede, ubestemte eller ufuldstændige resultater ved hjælp af VIASURE *SARS-CoV-2, influenza (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System, kan gentestning være påkrævet. Uløste resultater kan skyldes tilstedeværelsen af hæmmere i prøven eller forkert rehydrering af frysetørrede reaktionsblandingsrør. Hvis der opstår en instrumentfejl, kan det medføre ubestemmelige eller ufuldstændige resultater.

11. Kvalitetskontrol

VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System indeholder en intern kontrol i hvert *Flu A, Flu B & RSV* reaction tube og en endogen intern kontrol i hvert *SARS-CoV-2 (N1 + N2)* reaction tube, som bekræfter teknikkens korrekte funktion.

12. Ydelseskarakteristika

12.1. Klinisk sensitivitet og specificitet

Den kliniske præstation for VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System blev testet enkeltvis i hvert af de to reaktionsrør.

Den kliniske præstation for *Flu A, Flu B & RSV* reaction tube er blevet testet ved hjælp af 344 respiratoriske prøver (orofaryngeale podepinde) fra symptomatiske patienter. Disse resultater blev sammenlignet med dem, der blev opnået med en molekylær detektionsmetode (cobas® Influenza A/B & RSV (Roche)).

Resultaterne var følgende:

Flu A, B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	I alt
	+	157	2*	159
	-	7*	178	185
	I alt	164	180	344

Tabel 10. Sammenlignende resultater for influenza A.

Positiv procentvis overensstemmelse er >96 % og negativ procentvis overensstemmelse er >99 %.

**Den lave mængde skabelon-RNA i denne respiratoriske prøve er under detektionsgrænsen for den anvendte metode.*

Flu A, Flu B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	I alt
	+	99	4*	103
	-	1*	240	241
	I alt	100	244	344

Tabel 11. Sammenlignende resultater for influenza B.

Positiv procentvis overensstemmelse er >99 % og negativ procentvis overensstemmelse er >98 %.

**Den lave mængde skabelon-RNA i denne respiratoriske prøve er under detektionsgrænsen for den anvendte metode.*

Flu A, Flu B & RSV reaction tube	cobas® Influenza A/B & RSV (Roche)			
		+	-	I alt
	+	22	4*	26
	-	3*	315	318
	I alt	25	319	344

Tabel 12. Sammenlignende resultater for RSV.

Positiv procentvis overensstemmelse er >88% og negativ procentvis overensstemmelse er >99%.

*Den lave mængde skabelon-RNA i denne respiratoriske prøve er under detektionsgrænsen for den anvendte metode.

Den kliniske ydeevne af *SARS-CoV-2 (N1 + N2)* reaction tube blev testet ved hjælp af 254 luftvejsprøver (nasofaryngeale podepinde i Vircell Transport-medium) fra patienter med klinisk mistanke om COVID-19-sygdom eller andre lignende luftvejssygdomme. Resultaterne blev sammenlignet med dem, der blev opnået med den

kliniske diagnose udført med Simplexa™ COVID-19 Direct-analyse med diskrepanseanalyse udført i henhold til Charité-protokollen.

		Alternative RT-PCR-analyser		
		+	-	I alt
***SARS-CoV-2 (N1 + N2)* reaction tube**	+	63	2*	65
	-	0	189	189
	I alt	63	191	254

Tabel 13. Sammenlignende resultater for SARS-CoV-2.

*Indledende diagnose af en af de to prøver var ugyldig og blev rapporteret til patienten som positiv med henblik på forebyggelse og karantæneperiode.

SARS-CoV-2 (N1 + N2) reaction tube detekterede to positive prøver, der ikke blev detekteret ved hjælp af Simplexa™ COVID-19 Direct assay og Charité-protokollen.

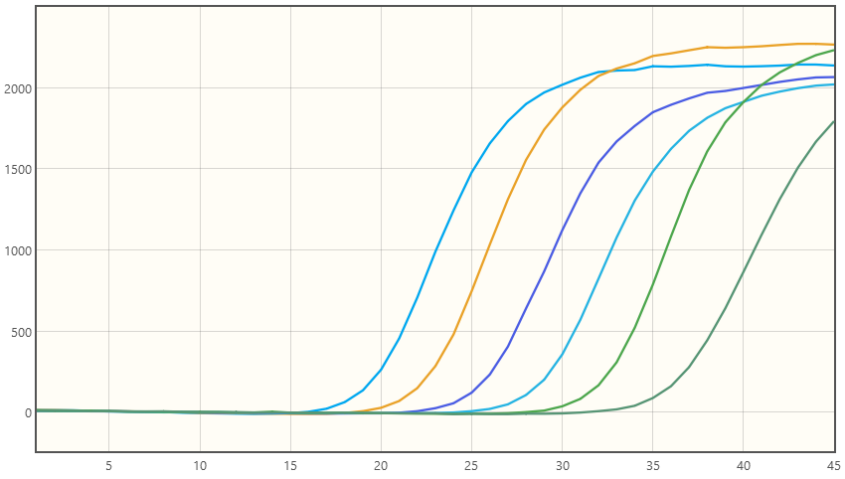
Den positive procentvise overensstemmelse (PPA) og den negative procentvise overensstemmelse (NPA) for *SARS-CoV-2 (N1 + N2)* reaction tube er henholdsvis >99 % og 98 %.

Resultaterne viser høj grad af overensstemmelse i påvisningen af SARS-CoV-2, influenza A, influenza B og/eller RSV -virus ved hjælp af VIASURE *SARS-CoV-2, influenza (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System.

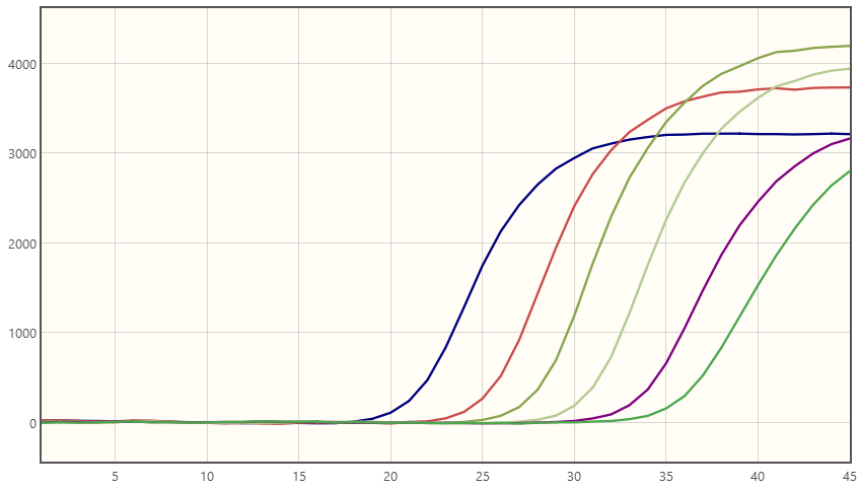
12.2. Analytisk sensitivitet

VIASURE *SARS-CoV-2, Influenza (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System har en detektionsgrænse på ≥ 10 genomkopier pr. reaktion for influenza A, ≥ 20 genomkopier pr. reaktion for influenza B, ≥ 2 genomkopier pr. reaktion for RSV og ≥ 5 genomkopier pr. reaktion for SARS-CoV-2 med en positiv rate på ≥ 95 % (figur 2, 3, 4, 5 og 6).

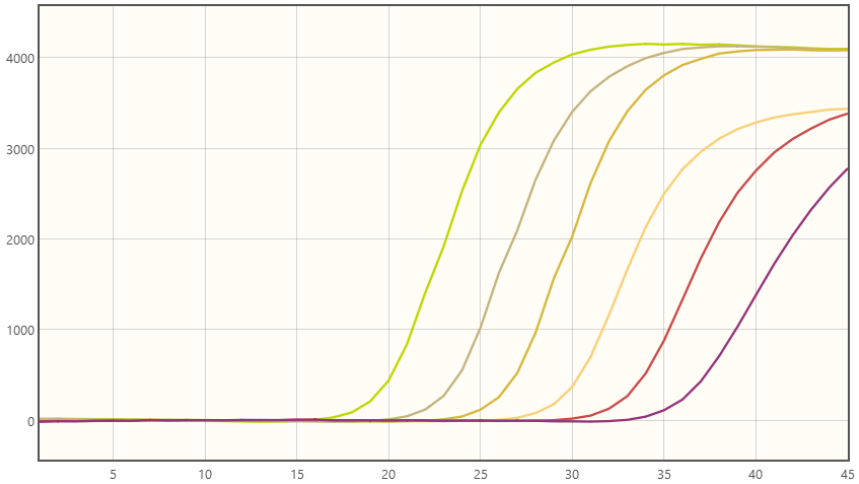
Figur 2. Fortyndingsserie for influenza A ($2*10^6$-$2*10^1$ kopier pr. reaktion) skabelon kørt på BD MAX™ System (kanalen 475/520 (FAM)).



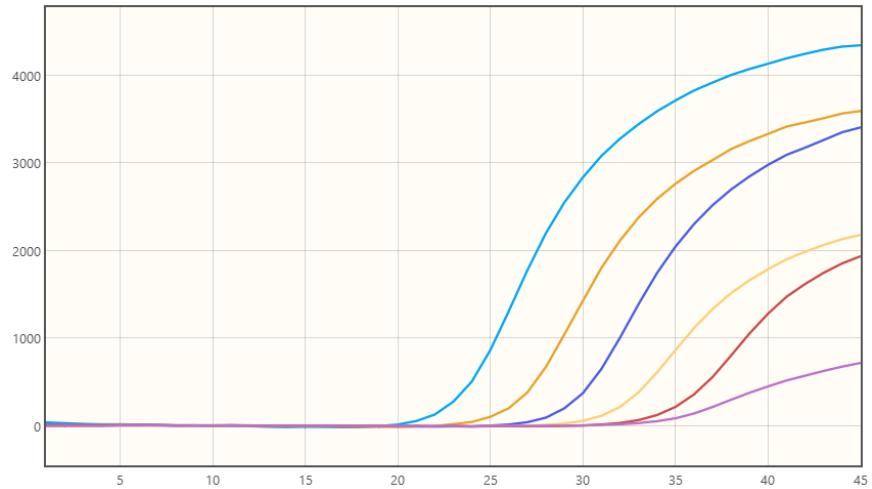
Figur 3. Fortyndingsserie af influenza-B ($2*10^6$-$2*10^1$ kopier pr. reaktion) skabelon kørt på BD MAX™ System (kanalen 585/630 (ROX)).



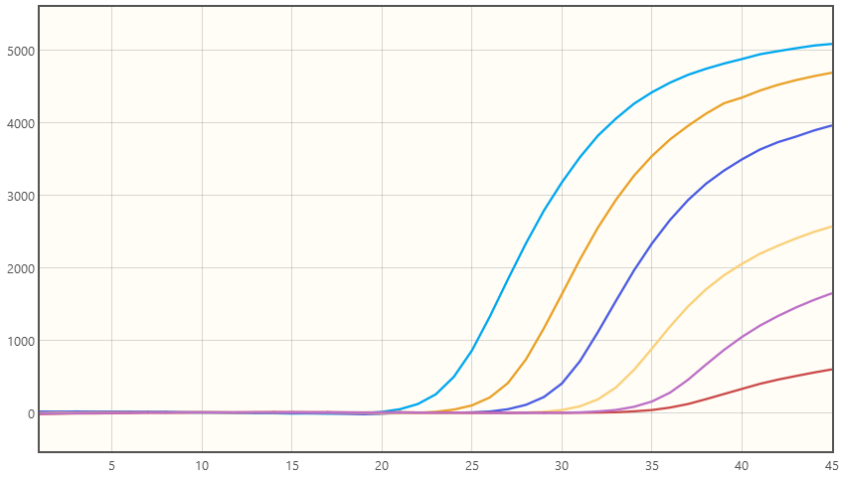
Figur 4. Fortyndingsserie af RSV ($2*10^6$-$2*10^1$ kopier pr. reaktion) skabelon kørt på BD MAX™ System (kanalen 630/665 (Cy5)).



Figur 5. Fortyndingsserie af SARS-CoV-2 *(N1+N2)* ($9,9*10^4$-$9,9*10^0$ og $5,0*10^0$ genomkopier pr. reaktion) skabelon kørt på BD MAX™-SYSTEMET (475/520 (FAM)-kanalen).



Figur 6. Fortyndingsserie af SARS-CoV-2 *(N1+N2)* (9,9*10⁴-9,9*10⁰ og 5,0*10⁰ genomkopier pr. reaktion) skabelon kørt på BD MAX™-systemet (630/665(Cy5)-kanalen).



12.3. Analytisk specificitet

Specificiteten af SARS-CoV-2-, influenza- (A+B)- og RSV-analysen blev bekræftet ved at teste et panel bestående af forskellige mikroorganismer, der repræsenterer de mest almindelige respiratoriske patogener. Der blev ikke påvist krydsreaktivitet mellem nogen af følgende testede mikroorganismer, undtagen for de målpatogenerne i hver analyse:

Krydsreaktivitetstest					
Human Adenovirus-type 1-5, 8, 15, 31, 40 og 41	-	Influenza A/Netherlands/398/2014 (H3N2)-virus (clade 3C.3a)	-/+	Influenza A/chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2)-virus	-/+
Bocavirus	-	Influenza A/Netherlands/2393/2015 (H3N2)-virus (clade 3C.2a)	-/+	Influenza A/Chicken/Myanmar/433/2016 (H9N2)-virus	-/+
Bordetella bronchiseptica	-	Influenza A/Newcastle/607/2019 (H3N2)-virus	-/+	Influenza A/Hong kong/1073/99 (H9N2)-virus	-/+
Bordetella holmesii	-	Influenza A/New York/39/2012 (H3N2)-virus	-/+	Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26-virus	-/+
Bordetella parapertussis	-	Influenza A/Ohio/2/2012 (H3N2)-virus	-/+	Influenza B/Brisbane/60/2008-virus	-/+
Bordetella pertussis	-	Influenza A/Perth/1001/2018 (H3N2)-virus	-/+	Influenza B/Colorado/6/2017-virus	-/+
Chlamydia caviae	-	Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2)-virus	-/+	Influenza B/Malaysia/2506/2004-virus	-/+
Chlamydia psittaci genotype A og C	-	Influenza A/South Australia/55/2014 (H3N2)-virus	-/+	Influenza B/Maryland/15/2016-virus	-/+
Chlamydophila pneumoniae CM-1	-	Influenza A/South Australia/55/2014, IVR-175 (H3N2)-virus	-/+	Influenza B/Netherlands/207/06-virus	-/+
Human coronavirus 229E OC43, NL63 og HKU1	-	Influenza A/Switzerland/9715293/2013 (H3N2)-virus	-/+	Influenza B/Netherlands/2518/2016 (clade 1A)-virus	-/+

Krydsreaktivitetstest					
MERS Coronavirus	-	Influenza A/Texas/50/2012 (H3N2)-virus	-/+	Influenza B/Nevada/3/2011-virus	-/+
SARS Coronavirus-stamme Frankfurt 1	-	Influenza A/Thüringen/5/2017 (H3N2)-virus (Clade 3C2a.1)	-/+	Influenza B/New Jersey/1/2012-virus	-/+
SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 p.1	-/+	Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C)-virus	-/+	Influenza B/Texas/02/2013-virus	-/+
SARS-CoV-2 strain 2019-nCoV/Italy-INMI1	-/+	Influenza A/Victoria/210/2009(H3N2)-virus	-/+	Influenza B/Townsville/8/2016-virus	-/+
SARS-CoV-2 isolate Australia/VIC01/2020	-/+	Influenza A/Victoria/361/2011 (H3N2)-virus	-/+	Influenza B/Canberra/11/2016-virus	-/+
SARS-CoV-2 isolate Wuhan-Hu-1	-/+	Influenza A/Victoria/361/2011 IVR-165 (H3N2)-virus	-/+	Influenza B/Florida/4/2006-virus	-/+
SARS-CoV-2 strain 2019nCoV/USAWA1/2020	-/+	Influenza A/Anhui/01/2005 (H5N1)-virus	-/+	Influenza B/Florida/07/2004-virus	-/+
Enterovirus 68 og 71	-	Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1)-virus	-/+	Influenza B/Guangdong/120/2000-virus	-/+
Enterovirus Echovirus 11 og 30	-	Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1)-virus	-/+	Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39)-virus	-/+
Enterovirus Coxsackievirus A24, A9 og B3	-	Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1)-virus	-/+	Influenza B/ Jiangsu/10/2003-virus	-/+
Haemophilus influenzae MinnA	-	Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a-virus	-/+	Influenza B/Massachusetts/2/2012-virus	-/+
Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09-virus	-/+	Influenza A/chicken/Yunnan/1251/2003 (H5N1)-virus	-/+	Influenza B/Netherlands/365/2016 (clade 3)-virus	-/+
Influenza A/California/7/2009(H1N1)pdm09-virus	-/+	Influenza A/common magpie/Hong Kong/645/2006 (H5N1)-virus	-/+	Influenza B/Phuket/3073/2013 virus	-/+
Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09-virus	-/+	Influenza A/duck/Hunan/795/2002 (H5N1)-virus	-/+	Influenza B/Texas/06/2011-virus	-/+
Influenza A/Massachusetts/15/2013 (H1N1)pdm09-virus	-/+	Influenza A/Egypt/321/2007 (H5N1)-virus	-/+	Influenza B/Wisconsin/1/2010-virus	-/+
Influenza A/Michigan/45/2015 (H1N1)pdm09-virus	-/+	Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1)-virus	-/+	Influenza B/Wisconsin/1/2010 BX-41A-virus	-/+
Influenza A/Netherlands/1250/2016 (H1N1)pdm09-virus (clade 6B.1)	-/+	Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1)-virus	-/+	*Legionella bozemanii*	-
Influenza A/New Caledonia/20/99(H1N1)-virus	-/+	Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29 -virus	-/+	*Legionella dumoffii*	-
Influenza A/New York/18/2009 (H1N1)pdm09-virus	-/+	Influenza A/Hong Kong/213/2003 (H5N1)-virus	-/+	*Legionella longbeachae*	-

Krydsreaktivitetstest					
Influenza A/Singapore/GP1908/2015, IVR-180 (H1N1)pdm09-virus	-/+	Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30-virus	-/+	*Legionella micdadei*	-
Influenza A/Sydney/134/2018 (H1N1)pdm09-virus	-/+	Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1)-virus	-/+	*Legionella pneumophila*	-
Influenza A/Victoria/2040/2018 (H1N1)pdm09-virus	-/+	Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1)-virus	-/+	Human metapneumovirus A og B	-
Influenza A/PR/8/34 (H1N1)-virus	-/+	Influenza A/Vietnam/1194/2004 (H5N1)-virus	-/+	*Moraxella catarrhalis*	-
Influenza A/Brisbane/117/2018 (H3N2)-virus	-/+	Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1)-virus	-/+	*Mycoplasma pneumoniae*	-
Influenza A/Brisbane/1028/2017 (H3N2)-virus	-/+	Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1)-virus	-/+	*Mycobacterium tuberculosis* ikke rifampin-resistent	-
Influenza A/Fujian/411/2002 (H3N2)-virus	-/+	Influenza A/Whooper Swan/R65/2006 (H5N1)-virus	-/+	Human parainfluenza 1, 2, 3 og 4 vira	-
Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2)-virus	-/+	Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4-virus	-/+	*Pneumocystis jirovecii* Type A1 og g885652	-
Influenza A/Hong Kong/4801/2014 (H3N2)-virus	-/+	Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3)-virus	-/+	Human rhinovirus type C	-
Influenza A/Hong Kong/4801/2014, NYMC X-263B (H3N2)-virus	-/+	Influenza A/Duck/Lao/XBY004/2014 (H5N6) (Clade 2.3.4.4)-virus	-/+	*Staphylococcus aureus* subsp. *aureus*	-
Influenza A/Indiana/8/2011 (H3N2)v virus	-/+	Influenza A/DE-SH/Reiherente/AR8444/2013 (H5N8)-virus	-/+	*Staphylococcus epidermidis*	-
Influenza A/Indiana/10/2011 (H3N2)v-virus	-/+	Influenza A/Turkey/Germany/R2485-86/2014 (H5N8)-virus	-/+	*Streptococcus pneumoniae* Z022	-
Influenza A/Kansas/14/2017 (H3N2)-virus	-/+	Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2)-virus	-/+	*Streptococcus pyogenes*	-
Influenza A/Kansas/14/2017, NYMC X-327 (H3N2)-virus	-/+	Influenza A/Mallard/Netherlands/2/2009 (H7N7)-virus	-/+	*Streptococcus salivarius*	-
Influenza A/Kumamoto/102/2002 (H3N2)-virus	-/+	Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1-virus	-/+	Respiratorisk syncytialvirus (RSV) A og B (stamme CH93(18)-18)	-/+
Influenza A/Minnesota/11/2010 (H3N2)v-virus	-/+	Influenza A/Anhui/1/2013 (H7N9)-virus	-/+	Human respiratorisk syncytialvirus, stamme, Long	-/+
Influenza A/Minnesota/11/2010 X203 (H3N2)v-virus	-/+	Influenza A/Guangdong/17SF003/2016 (H7N9)-virus	-/+		

Tabel 14. Reference patogene mikroorganismer, som anvendes i denne undersøgelse.

12.4. Analytisk reaktivitet

Reaktiviteten af VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **SARS-CoV-2** blev evalueret mod RNA fra Human 2019-nCoV-stamme BetaCoV/Germany/BavPat1/2020 p.1, Human

2019-nCoV-stamme 2019-nCoV/Italy-INMI1, SARS-CoV-2 stamme 2019nCoV/USA-WA1/2020, syntetiske RNA-kontroller for to varianter af SARS-CoV-2-virus: MT007544.1 (SARS-CoV2-isolat Australia/VIC01/2020) og MN908947.3 (SARS-CoV-2-isolat Wuhan-Hu-1) med positivt resultat.

Reaktiviteten ved VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza A** blev evalueret mod RNA ekstraheret fra følgende stammer: Influenza A/Brisbane/02/2018, IVR-190 (H1N1)pdm09-virus, Influenza A/California/7/2009(H1N1)pdm09-virus, Influenza A/Dominican Republic/7293/2013 (H1N1)pdm09-virus, Influenza A/Massachusetts/15/2013 (H1N1)pdm09-virus, Influenza A/Michigan/45/2015 (H1N1)pdm09-virus, Influenza A/Netherlands/1250/2016 (H1N1)pdm09-virus (clade 6B.1), Influenza A/New Caledonia/20/99(H1N1)-virus, Influenza A/New York/18/2009 (H1N1)pdm09-virus, Influenza A/Singapore/GP1908/2015-virus, IVR-180 (H1N1)pdm09-virus, Influenza A/Sydney/134/2018 (H1N1)pdm09-virus, Influenza A/Victoria/2040/2018 (H1N1)pdm09-virus, Influenza A/PR/8/34 (H1N1)-virus, Influenza A/Brisbane/117/2018 (H3N2)-virus, Influenza A/Brisbane/1028/2017 (H3N2)-virus, Influenza A/Fujian/411/2002 (H3N2)-virus, Influenza A/Hiroshima//52/2005 (IVR-142) (H3N2)-virus, Influenza A/Hong Kong/4801/2014 (H3N2)-virus, Influenza A/Hong Kong/4801/2014 NYMC X-263B (H3N2)-virus, Influenza A/Indiana/8/2011 (H3N2)v-virus, Influenza A/Indiana/10/2011 (H3N2)v-virus, Influenza A/Kansas/14/2017 (H3N2)-virus, Influenza A/Kansas/14/2017, NYMC X-327 (H3N2)-virus, Influenza A/Kumamoto/102/2002 (H3N2)-virus, Influenza A/Minnesota/11/2010 (H3N2)v virus, Influenza A/Minnesota/11/2010 X203 (H3N2)v-virus, Influenza A/Netherlands/398/2014 (H3N2)-virus (clade 3C.3a), Influenza A/Netherlands/2393/2015 (H3N2)-virus (clade 3C.2a), Influenza A/Newcastle/607/2019 (H3N2)-virus, Influenza A/New York/39/2012 (H3N2)-virus, Influenza A/Ohio/2/2012 (H3N2)-virus, Influenza A/Perth/1001/2018 (H3N2)-virus, Influenza A/Singapore/INFIMH-16-0019/2016 (H3N2)-virus, Influenza A/South Australia/55/2014 (H3N2)-virus, Influenza A/South Australia/55/2014, IVR-175 (H3N2)-virus, Influenza A/Switzerland/9715293/2013 (H3N2)-virus, Influenza A/Texas/50/2012 (H3N2)-virus, Influenza A/Thüringen/5/2017 (H3N2)-virus (Clade 3C2a.1), Influenza A/Uruguay/716/2007 (H3N2)(NYMC X-175C)-virus, Influenza A/Victoria/210/2009(H3N2)-virus, Influenza A/Victoria/361/2011 (H3N2)-virus, Influenza A/Victoria/361/2011 IVR-165 (H3N2)-virus, Influenza A/Anhui/01/2005 (H5N1)-virus, Influenza A/Anhui/01/2005 x PR8-IBCDC-RG6 (H5N1)-virus, Influenza A/chicken/Vietnam/NCVD-016/2008 (H5N1)-virus, Influenza A/chicken/Vietnam/NCVD-016/2008 x PR8-IDCDC-RG12 (H5N1)-virus, Influenza A/chicken/Vietnam/NCVD-03/08 (H5N1) - PR8-IDCDC-RG25a virus, Influenza A/chicken/Yunnan/1251/2003 (H5N1)-virus, Influenza A/common magpie/Hong Kong/645/2006 (H5N1)-virus, Influenza A/duck/Hunan/795/2002 (H5N1)-virus, Influenza A/Egypt/321/2007 (H5N1)-virus, Influenza A/Egypt/321/2007 x PR8-IDCDC-RG11 (H5N1)-virus, Influenza A/Egypt/3300-NAMRU3/2008 x PR8-IDCDC-RG13 (H5N1)-virus, Influenza A/Egypt/N03072/2010 (H5N1) x PR8-IDCDC-RG29-virus, Influenza A/Hong Kong/213/2003 (H5N1)-virus, Influenza A/Hubei/1/2010 (H5N1) x PR8-IDCDCRG30 virus, Influenza A/India/NIV/2006 xPR8-IBCDC-RG7 (H5N1)-virus, Influenza A/Japanese white eye/Hong Kong/1038/2006 (H5N1)-virus, Influenza A/Vietnam/1194/2004 (H5N1)-virus, Influenza A/Vietnam/1194/2004 (NIBRG-14) (H5N1)-virus, Influenza A/Vietnam/1203/2004 x PR8-IBCDC-RG (H5N1)-virus, Influenza A/Whooper Swan/R65/2006 (H5N1)-virus, Influenza A/pheasant/New Jersey/1355/1998 (H5N2)-PR8-IBCDC-4 virus, Influenza A/Duck/Singapore-Q/F119-3/97 (H5N3)-virus, Influenza A/Duck/Lao/XBY004/2014 (H5N6)-virus (Clade 2.3.4.4), Influenza A/DE-SH/Reiherente/AR8444/ 2016 (H5N8)-virus, Influenza A/Turkey/Germany/R2485-86/2014 (H5N8)-virus, Influenza A/turkey/Virginia/2002 x PR8-IBCDC-5 (H7N2)-virus, Influenza A/Mallard/Netherlands/2/2009 (H7N7)-virus, Influenza A/Mallard/Netherlands/12/2000 (H7N7) - IBCDC-1-virus, Influenza A/Anhui/1/2013 (H7N9)-virus, Influenza A/Guangdong/17SF003/2016 (H7N9)-virus, Influenza A/Chicken/Hong Kong/G9/1997 x PR8-IBCDC-2 (H9N2)-virus,

Influenza A/Chicken/Myanmar/433/2016 (H9N2)-virus, Influenza A/Hong Kong/1073/99 (H9N2)-virus, Influenza A/Hong Kong/33982/2009 (H9N2) x PR8-IDCDC-RG26-virus, der viser positive resultater.

Reaktiviteten af VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™ System for **Influenza B** blev evalueret mod RNA ekstraheret fra følgende stammer: Influenza B/Brisbane/60/2008-virus, Influenza B/Colorado/6/2017-virus, Influenza B/Malaysia/2506/2004-virus, Influenza B/Maryland/15/2016-virus, Influenza B/Netherlands/207/06-virus, Influenza B/Netherlands/2518/2016 (clade 1A)-virus, Influenza B/Nevada/3/2011-virus, Influenza B/New Jersey/1/2012-virus, Influenza B/Texas/02/2013-virus , Influenza B/Townsville/8/2016-virus (**B/Victoria lineage**); Influenza B/Canberra/11/2016-virus, Influenza B/Florida/4/2006-virus, Influenza B/Florida/07/2004-virus, Influenza B/Guangdong/120/2000-virus, Influenza B/Hubei Wujiagang/158/2009 (NYMC BX-39)-virus, Influenza B/Jiangsu/10/2003-virus, Influenza B/Massachusetts/2/2012-virus, Influenza B/Netherlands/365/2016 (clade 3)-virus, Influenza B/Phuket/3073/2013-virus, Influenza B/Texas/06/2011-virus, Influenza B/Wisconsin/1/2010-virus, Influenza B/Wisconsin/1/2010 BX-41A-virus (**B/Yamagata lineage**), der viser et positivt resultat.

Reaktiviteten af VIASURE *SARS-CoV-2, Flu (A+B) & RSV* Real Time PCR Detection Kit for BD MAX™-System for **RSV** blev bekræftet mod RNA ekstraheret fra RSV A og B (stamme CH93 (18)-18) og Human respiratorisk syncytialvirus, stamme Long, viser positivt resultat.

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14. Symbols for IVD components and reagents/Symboler for IVD-komponenter og -reagenser

IVD	*In vitro* diagnostic device *In vitro*-diagnostisk udstyr		Keep dry Opbevares tørt		Use by Anvendes inden		Manufacturer Producent
LOT	Batch code (Lot) Batch-kode (parti)		Consult Instructions for Use Se brugsanvisning		Temperature limitation Temperaturbegrænsning	Σ	Contains sufficient for <n> test Indeholder nok til <n> tests
DIL	Sample diluent Prøvefortynding	REF	Catalognumber Katalognummer				

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